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William Jesse Yawney
Iowa State University

Yawney, William Jesse

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Anatomy and physiology of sugar maple (*Acer saccharum* Marsh) seedlings uninoculated or inoculated with the vesicular-arbuscular endomycorrhizal fungus *Glomus etunicatum*

by

William Jesse Yawney

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GENERAL INTRODUCTION

It has been demonstrated that vesicular-arbuscular endomycorrhizal (VAM) symbiosis is a natural and integral part of the physiology of a wide variety of tree species (Fardelmann and McNabb, 1981; Guttay, 1982; Kormanik et al., 1976 and 1977; Levy and Krikun, 1980; Pope et al., 1983; Riffle, 1980; Schultz et al., 1981; Verkade and Hamilton, 1983a and 1983b). Sugar maple (Acer saccharum Marsh) is one of the most important tree species in North America and has been known to form the symbiosis since 1914 (McDougall, 1914). The importance of the symbiosis to this species has not been investigated, however.

The distribution of sugar maple (Acer saccharum Marsh) is limited by a broad yet definite set of environmental characteristics (Post, 1968). Temperature limits distribution to the north and south. Soil physical characteristics are particularly important, especially those that determine soil moisture. Although very little specific information is available, sugar maple is known to require high nutrition. Attempts to establish sugar maple artificially have generally met with complete failure (Yawney, 1968). Planting failures have been attributed to the selection of poor sites, weed competition, frost damage and animal damage. Growth rate is the limiting factor in nursery production where 3 or 4 seasons are often required to produce a plantable seedling (Wood and

Hanover, 1981).

Webb (1976) investigated the effects of light intensity and photoperiod on root elongation rates and found that active root growth proceeded at the expense of shoot growth followed by a period of increased shoot growth and reduced root growth. The cyclic nature of these processes allowed for the maintenance of a relatively stable root to shoot ratio. Reduction in light intensity to a point that limited photosynthesis severely inhibited root growth, while a return to adequate light intensities resulted in normal rates of growth. It appeared that root growth was dependent on photosynthate supply. During a period of inactivity, roots became suberized. This was followed by the growth of white roots from these suberized tips during an active period. Taylor and Dumbroff (1975) and Dumbroff and Brown (1976) found that root growth in sugar maple continued through the winter ceasing only when the ground was frozen in December and January. A surge of root growth was observed in late February to early April indicating that this species prepares for spring growth very early in the year. In fact, sugar maple seeds are often observed to germinate under snow cover in early spring. An increase in cytokinin activity, just before bud break, followed this early root activity. It was suggested that synthesis of cytokinins is not the initial step leading to the breaking of shoot dormancy since cytokinin

activity appeared after transition of the buds from the dormant to the quiescent state.

These physiological studies have been few in number and have ignored VAM symbiosis. Kessler (1966), using light microscopy, provided an anatomical description of VAM sugar maple roots collected from the field. Penetration of the root through an appressorium and the formation of coils, arbuscules and vesicles were observed. Hyphal constrictions were noted at various places where hyphae passed through cell walls. Kessler also noted a close association between fungal hyphae and host cell nuclei. A schematic diagram was included which indicated arbusculation in the cortical layers closest to the steele, while outer cortical layers contained primarily coils. This did not appear to be the case in the micrographs presented, however. Kessler concluded that the beaded root morphology expressed by sugar maple is not the result of mycorrhizal infection as previously believed. Rather, it appeared to be related to alternately favorable and unfavorable soil moisture conditions since the phenomenon was not observed deep in the soil or in hollows but was restricted to surface layers and hummocks. In 1972, Kessler and Blank reported the association of Endogone sporocarps with sugar maple but the species of fungi was not identified.

During the past 30 years, intensive research has established a general model that portrays VAM symbiosis as a

highly dynamic physiological interaction. Nearly all aspects of the host plant's physiology are affected. The effect that has received the most attention has been the improved growth of plants with the symbiosis over nonmycorrhizal plants in media deficient in available phosphorus (P). The P levels at which this phenomenon is expressed are quite common in the natural environment, as are these fungi, which have been characterized as being ubiquitous.

Mycorrhizal plants, while deriving P from the same source, the soil solution (Hayman and Mosse, 1972; Ross and Gilliam, 1973; Sanders and Tinker, 1973; Rhodes and Gerdemann, 1975; Tinker, 1975; Barrow et al., 1977), are able to achieve optimal P nutrition. This phenomenon results from the exploitation of a significantly greater soil volume due to the increased surface area represented by extramatrical VAM hyphae. The significance of the effect is due to the extremely slow rate of P diffusion in the soil solution that results in the rapid formation of P depletion zones immediately adjacent to host roots. Translocation ^{32}P, by VAM hyphae has been shown to occur over a distance of 7 cm from the host root (Rhodes and Gerdemann, 1975). As one might expect, plants with relatively fine roots generally exhibit less of a mycorrhizal response than coarse rooted plants in P deficient media (Crush, 1974; Baylis, 1972; Johnson, 1976; St. John, 1980). In addition, Cress et al. (1979) reported that

VAM hyphae possess a significantly greater affinity for soil P than onion roots. Earlier evidence that VAM hyphae are able to dissolve sparingly soluble forms of P, e.g., rock phosphate (Murdoch et al., 1967; Mosse, 1973; Azcon et al., 1976), is now interpreted in terms of physical mechanisms. The hyphae have greater access to what P is released by the sparingly soluble sources.

Mechanisms for P uptake and transport by VAM hyphae were theorized by Woolhouse (1975). Woolhouse suggested an active uptake of P in the form of orthophosphate by extramatrical hyphae; requiring the use of a binding protein, passive transport out of the fungus at the arbuscule, active uptake of P across the host plasmalemma, passive loss of carbohydrate by the host cell and active uptake of the carbohydrate by the fungus. Once inside the extramatrical hyphae, orthophosphate is packaged in polyphosphate granules for transport. Electron dense granular bodies in the vacuoles of fungal hyphae have, in fact, been shown to consist of polyphosphate by X-ray microanalysis (Ling-Lee et al., 1975; Schoknecht and Hattingh, 1976). Polyphosphate content in the hyphae of onion mycorrhizae was measured at 0.03 gm cm^{-3} by X-ray microanalysis of cytochemically demonstrated polyphosphate (Cox et al., 1980). Using steroscopic high-voltage electron microscopy the authors determined that polyphosphate occupied 0.8% of the fungal volume. This information, combined with

cytoplasmic streaming rates of 12.6 cm h^{-1} observed with light microscopy resulted in a calculated flux rate of 2.7×10^{8} mol P $cm^{-2}s^{-1}$ that is sufficient to account for the increased phosphorus nutrition observed in mycorrhizal onion. Cooper and Tinker (1981) found that temperature and transpiration rate of the plant significantly influenced the rate of phosphorus transport in clover. In addition, evidence for cytoplasmic streaming as the mode of transport was presented since the cytoplasmic streaming inhibitor cytochalasin B effectively prevented P transport by the fungus.

The active arbuscule is believed to be the destination of fungal polyphosphate. Arbuscules are ideally suited for exchange between the fungus and host, due primarily, to the high surface area represented by these dichotomously branched fungal structures which are in intimate association with the host plasmalemma. To afford this close association, significant increases in the synthesis of host cell cytoplasm and plasmalemma are observed to coincide with arbuscule formation (Cox and Tinker, 1976). Cox and Tinker (1976) and White and Brown (1979) concluded that the living arbuscule is the site of active transfer between the host and endophyte due to the absence of polyphosphate granules in newly senescing arbuscules as well as the lack of host and endophyte organelles that are present when an arbuscule is fully formed.

Considerable amounts of mycorrhiza-specific alkaline

phosphatase has been demonstrated in active VAM infections (Gianinazzi-Pearson and Gianinazzi, 1978). The activity of the enzyme reached a peak in young, highly arbuscular infections. Gianinazzi and Gianinazzi-Pearson (1979) observed very intense alkaline phosphatase activity localized in the vacuoles of mature arbuscules and intercellular hyphae. The distribution of the enzyme mirrored that of vacuolar polyphosphate suggesting a role of alkaline phosphatase in the metabolic turnover of polyphosphate. The association of acid phosphatase activity with the organelles of immature arbuscular tips suggests a role of this enzyme in hyphal growth and arbuscular development. Intense acid phosphatase activity has also been demonstrated in the actively growing germ tubes of Glomus mosseae spores (MacDonald and Lewis, 1978).

The uptake of other elements has also been shown to be improved by VAM symbiosis. Cooper and Tinker (1978), using a split-plate culture system that allowed VAM hyphae to pass through a root barrier, observed the hyphal translocation of labelled Zn and S to the research plants. This was substantiated by Rhodes and Gerdemann (1980). There is evidence that VAM infection can increase the host's uptake of Cu (Timmer and Leyden, 1980) and evidence of a growth response due to increased Cu uptake (Gildon and Tinker, 1983). Jensen (1982) found increased uptake of Cu and Zn in barley. Calcium-45, injected into soil 4.5 cm away from host root

surfaces, was found in mycorrhizal but not nonmycorrhizal onion plants indicating hyphal transport of this element as well (Rhodes and Gerdemann, 1978). Ames et al. (1983) used a split culture system to demonstrate hyphal uptake of N in celery plants. Nitrogen appears to be important in the function of the symbiosis. Hall et al. (1984) suggested that applied N resulted in more efficient mycorrhizal uptake of P and Cu. Increasing rates of N fertilization resulted in higher levels of infection in lettuce roots (Hepper, 1983). This effect was observed at three P levels. At low P, the level of infection was highly dependent upon N level illustrating the potential for complex interactions.

An interesting interaction is the effect of mycorrhizal symbiosis on nitrogen fixation. Nitrogen fixation is increased by VAM infection in P deficient media (Mosse et al., 1976; Smith and Daft, 1977; Smith et al., 1979; Munns and Mosse, 1980). This response is believed to represent an indirect effect of the symbiosis. With adequate P nutrition, the plant is able to provide a more suitable environment for the N fixing symbiont.

Of great importance to plant growth and development in hostile environments is the effect of VAM fungi on plant water relations. Under drought conditions, one would expect that a mycorrhizal root system would have greater access to the limited soil moisture than a nonmycorrhizal root system due to

the surface area represented by extramatrical hyphae. An important factor in this regard could be the relatively small diameter of fungal hyphae relative to host roots. Nelson and Safir (1982) recorded higher water potentials, greater transpiration rates, higher hydraulic conductivities and lower leaf resistances in mycorrhizal onion plants. These responses were observed at low P but disappeared at high P and were therefore attributed in part to improved P nutrition. Levy and Krikun (1980) suggested that improved recovery from water stress of mycorrhizal rough lemon seedlings was due to an effect of the fungus on stomatal regulation rather than on root resistance. The hydraulic conductivities of the root systems of red clover were two to three times higher per unit length of root for mycorrhizal plants in P deficient soil (Hardie and Leyton, 1981). This was attributed to the greater surface area of the symbiotic root system. Leaf resistances were reduced by the symbiosis in Boutelona gracilis in an unfertilized potting soil (Allen, 1982). Under conditions of high moisture tension, a species of range land grass had 47% lower leaf resistance with VAM infection (Stahl and Smith, 1984). Measuring soil water potential is difficult and complex and more evidence is needed to define the mechanisms responsible. When considering the difficulties involved in quantifying soil water potential and the fact that nutritional responses interact with the mycorrhizal effect on water

uptake, it is clear that more work is required in this area to clearly define the mechanisms responsible for the observed responses. It does appear possible however, that improved water status may be a more important part of the mycorrhizal response than P nutrition under conditions of high moisture stress.

There are indications that VAM infections can alter plant hormone balances. Levy and Krikun (1980) postulated that mycorrhizal symbiosis affects plant water relations through its affect on root-shoot hormone balance. Sweetgum seedlings have been shown to grow better with mycorrhizal infection even when the controls have adequate soil P and N, indicating the existence of another effect that could be hormone related (Schultz et al., 1979). Dormancy in first year yellow poplar cuttings was negated by VAM infection suggesting a possible hormone triggering mechanism (Kormanik et al., 1979). Allen et al. (1980) provided direct evidence for the alteration of cytokinin levels in Bouteloua gracilis. In view of the similarities between mycorrhizal and pathogenic infections and the well-known effects of the latter on plant hormone levels, one might also expect an effect of the symbiosis on hormone levels. Clearly, this is a complex area that involves relative levels of various hormones and one that needs further investigation of a factorial nature.

In his review article, Hayman (1983) discusses other

possible influences of VAM infection on host plant physiology. These include resistance to pathogenic fungi by decreasing the leakiness of cells, modification of root exudation and, therefore, modification of rhizosphere organism populations, and increases in the levels of chlorophyll and arginine in the host plant.

Mycorrhizal infection results in a reciprocal interaction where benefit to the plant is accompanied by a carbohydrate drain on the host by the fungus (Harley, 1975). Oil droplets have been commonly observed in hyphae and spores of VAM fungi (Mosse, 1959; Mosse and Bowen, 1968; Ho and Trappe, 1973; Cooper, 1976). Ho and Trappe (1973) reported the accumulation of ^{14}C in Glomus mosseae spores formed after exposure of the host to $^{14}CO_2$. Cox et al. (1975) demonstrated a concentration of ^{14}C in lipid-rich hyphae and vesicles in mycorrhizal roots using electron microscopy autoradiography. Reduction in the dry weight of mycorrhizal but not nonmycorrhizal tomato plants subjected to photosynthesis disrupting ozone stress is interpreted by McCool and Menge (1983) as evidence of a fungal carbohydrate drain. Losel and Cooper (1979) using ^{14}C, with intact plants or detached roots, noted that carbon incorporation by onion roots from exogenous sucrose, acetate or glycerol as well as from translocated photosynthate, was increased significantly by VAM infection. This greater accumulation by mycorrhizal onion may have reflected greater

rates of photosynthesis due to the symbiosis.

There is no evidence of conversion of host photosynthate into the fungal carbohydrates trehalose and mannitol with VAM systems, unlike ectomycorrhizae (Hayman, 1974). Cooper and Losel (1978) studied the distribution, quantity and composition of lipid in VAM onion, clover and ryegrass, microscopically and biochemically. Mycorrhizal roots had significantly higher levels of total lipid and triglyceride than nonmycorrhizal roots. High levels of neutral lipids including triglyceride, diglyceride and free fatty acids occurred in extramatrical mycelium. Higher levels of polar lipids, including diphosphatidyl glycerol, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidycholine, phosphatidyl glycerol, phosphatidic acid, and phosphatidyl inositol, were found in VAM onion roots and all but the latter two were significant components of VAM hyphae.

It is difficult to determine the significance of the fungal photosynthate drain, since mycorrhizal hyphae make up only a small portion of a mycorrhizal root system. Hayman (1983) mentioned an average figure of 10% or less for the weight of the fungal component of mycorrhizal roots. A figure of 1% of the total plant weight was estimated for hyphae of Glomus mosseae in association with clover roots (Bevege et al., 1975). From chitin assays, it was estimated that the endophyte comprised up to 9% and 17% of the weight of onion

and clover roots, respectively (Hepper, 1977). Hayman (1983) points out that observed reductions in growth of mycorrhizal plants under stress, e.g., shade or ozone, compared to nonmycorrhizal plants supplied with adequate P were too large to be accounted for by the carbon drain of an endophyte that comprises such a small percentage of the plant weight. He feels that it can be explained by impaired P uptake by the mycorrhizal fungus due to impairment of the fungal carbon source by the stress. Smaller mycorrhizal leek plants contained higher levels of P than P fertilized nonmycorrhizal leeks supporting the idea of a significant photosynthate drain (Stribley et al., 1980). Snellgrove et al. (1982), using $^{14}CO_2$, estimated that 7% more of the total carbon fixed by leek plants was transferred to the below ground portion when the fungus was present. The VAM leeks, again, contained higher levels of P than nonmycorrhizal leeks that were supplied with sufficient P to achieve similar growth. Conversely, concentrations of P in larger VAM sweetgum seedlings were half that of nonmycorrhizal seedlings (Schultz et al., 1979). The latter situation could have involved a more efficient endophyte.

Pang and Paul (1980) exposed mycorrhizal and nonmycorrhizal nodulated faba beans to $^{14}CO_2$ and $^{15}N_2$ above and below ground, respectively. Mycorrhizal and nonmycorrhizal plants fixed similar amounts of N and produced

similar amounts of dry weight. There was a difference in distribution of fixed ^{14}C however with 37% transferred to the below ground portion of the nonmycorrhizal beans as compared to 47% for infected plants. Most of this difference was attributed to below ground respiration since significantly greater quantities of $^{14}CO_2$ were evolved in the mycorrhizal systems. In another study with faba beans, it was estimated that VAM fungi used approximately 4% of the carbon fixed by the host plants (Kucey and Paul, 1982). Faba bean nodules used 6% and 12% of the available fixed carbon in nonmycorrhizal and VAM plants, respectively. This was attributed to the greater nodule size that resulted from improved mineral nutrition in the VAM hosts. Under P nutrition conditions, that provided ample P for nonmycorrhizal ryegrass plants, VAM and nonmycorrhizal plants had equal levels of P in their tissues but VAM plants had significantly reduced top weights (Buwalda and Goh, 1982). In addition, VAM plants had lower total oxidizable C, lower soluble sugar content, a lower C/N ratio and higher free NH_4^+ levels. The authors attributed the latter to impaired protein synthesis that could have resulted from an inhibited carbon supply.

Bethlenfalvay et al. (1982) attributed stunting observed during the first nine weeks of growth with soybeans to a carbohydrate demand by the endophyte when the shoot-to-root ratio and photosynthetic capacity of the host were low. This

was reversed, resulting in larger VAM plants at week 15. After one growing season in fumigated nursery soil, VAM silver maple seedlings at high P were 20% smaller in dry weight than nonmycorrhizal seedlings also grown at high P (Schultz et al., 1982). This was interpreted to represent a carbohydrate drain by the endophyte under conditions where its presence is not required for P uptake resulting in a growth depression.

Therefore, it is difficult to assess the significance of the fungal drain. Many interacting factors have a role in determining carbon balances. Certainly, different plant types, e.g., herbaceous versus woody plants, ones that have below ground carbohydrate sinks in the form of bulbs or tubers or ones that form associations with N fixing bacteria or actinomycetes, will have different responses to a particular symbiont. In fact, a particular species or isolate of VAM fungi may be more efficient in the uptake of P per photosynthate energy used.

The degree of intimacy expressed between the two organisms involved in a VAM symbiosis is so great as to preclude separation of discrete effects on the physiology of either. The site of intimacy of host-endophyte associations is the arbuscule (Kinden and Brown, 1975a). Infection begins at the root surface with the formation of an appressorium like structure and subsequent penetration between epidermal cells (Kessler, 1966; Carling and Brown, 1982).

Colonization of active feeder roots occurs in the zone of differentiation and elongation and is restricted to host cortical cells (Carling and Brown, 1982). With the initiation of secondary growth, host cortical cells begin to die and are sloughed off ending infection in that area of the root. The infection must, therefore, be extremely dynamic in order to keep pace with a habitat that is continually changing its location. Colonization resulting from one entry point is considered a single infection unit and is characterized by two infection fronts that move longitudinally in the root in opposite directions from the entry point (Gallaud, 1905; Cox and Sanders, 1974). An infection unit represents an age sequence with the oldest part of the infection near the entry point. In advanced infections, infection units are often seen to overlap making a determination of the sequence of events impossible. The formation of new infection units occurs from runner hyphae that grow along the outer surface of the root, penetrating the root at various points. A mature infection may contain from two to twenty hyphal entry points per cm of root (Mosse, 1959; Sanders and Tinker, 1973). Internal spread of infection units occurs intercellularly by possible fungal modification of the host middle lamella and intracellularly, from cell to cell (Scannerini and Bonfante-Fasolo, 1983). Hyphal constrictions are observed at cell wall entry points (Kinden and Brown, 1975b). Kinden and Brown (1975c) noted

that the middle lamella had a modified, granular appearance at the point of entry in their electron micrographs and suggested that cell wall penetration, may be enzymatically mediated as well as mechanical. Intercellular hyphae normally have diameters of 3 to 30 μm in coarse endophytes and less than 2 μm in fine endophytes (Gianinazzi-Pearson et al., 1981).

Intracellular hyphae may continue through the cell unchanged or may alter their shape to form vesicles, coils or arbuscules (Kinden and Brown, 1975a). Vesicles are thick walled lipid storage organs that often obtain the shape of the cortical cells they occupy (Kinden and Brown, 1975b; Strullu et al., 1983). A terminate coiling of intracellular hyphae produces a coil. Arbuscules are formed, when, upon entry through the cell wall, the hyphae branch repeatedly in a dichotomous fashion until, in some cases, the entire cell volume is occupied with short bifurcate hyphal branches similar in diameter to host mitochondria (0.3 to 0.5 μm). At the point of entry, a "collar" of material, similar in appearance to the host cell wall, surrounds the trunk of the arbuscule (Cox and Sanders, 1974; Kariya and Toth, 1981). This layer stains lightly with OsO_4 relative to the fungal wall it surrounds and appears to have a random microfibril arrangement in an unstained matrix. The collar thins with distance from the cell wall along the arbuscule trunk and is not observed after the first hyphal branching occurs. The

host plasmalemma maintains its integrity during arbuscule formation, proliferating to afford intimate contact with each arbuscule branch or tip. Cox and Tinker (1976) analyzed tracings of electron micrographs of mycorrhizal onion roots with an image analyzing computer and calculated increases in host plasmalemma surface area and host cytoplasm volume of 3 and 23 fold, respectively. Considering the extent of the surface area involved in this interaction, the suitability of the arbuscule for exchange is apparent.

Sward (1981) detected chitin in the walls of VAM spores histochemically. Nemec (1981) demonstrated the presence of chitin in the walls of intercellular hyphae and vesicles. Intercellular hyphae, as well as the larger intracellular hyphae, contain numerous discrete vacuoles that almost fill the cell lumen resulting in small peripheral areas of fungal cytoplasm (Cox and Sanders, 1974). The fungal cytoplasm in these hyphae contain numerous organelles such as nuclei and mitochondria (Gianinazzi-Pearson et al., 1981).

Conversely, the arbuscule wall has osmiophillic and acidic properties as well as PAS reactivity, suggesting that it is composed primarily of glycolipid (Nemec, 1981). Bonfante-Fasolo (1982) demonstrate the presence of N-acetyl-glucosamine in arbuscule walls by the distribution of wheat germ agglutinin. They discussed the possibility of a gap between chitin subunit formation and polymerization into

fibrils. The lack of a chitinous wall is another indication that transfer occurs at the arbuscule. In addition, sphingolipids, which are known to function in membrane ion transport, were one of the glycolipids demonstrated by Nemec (1981). Arbuscular hyphae are densely cytoplasmic at maturity and contain numerous nuclei, mitochondria and other organelles needed for normal metabolism (Kinden and Brown, 1975c). Numerous small vacuoles, some lipid droplets and glycogen particles are also observed, the latter being more abundant in larger hyphal branches. Hyphal vacuoles throughout the fungal network contain polyphosphate (Ling-Lee et al., 1975; White and Brown, 1979).

Arbuscules, as mentioned, are extremely short-lived structures. Kinden and Brown (1975) described the changes in arbuscules that occur as they senesce. A disorganization of cytoplasmic structure is followed by cytoplasmic disintegration and the collapsing of fungal walls. The collapsed branches coalesce, forming dense aggregate clumps. The detoriation progresses from the fine arbuscular tips toward the arbuscular trunk resulting in a single remnant mass near the point of entry.

The arbuscule branches and host plasmalemma are not in direct contact but are separated by an interfacial region. Two distinct sub-regions are commonly observed. The area on the fungal side of the region is occupied by a granular and

(or) fibrillar electron dense matrix. The plasmalemma side of the region is seen as an electron lucent space devoid of substance. Carling et al. (1977), however, demonstrated that the translucent space was an artifact of fixation since simultaneous glutaralhyde-osmium tetroxide fixation preserved a granular matrix throughout the interfacial zone. Since the material surrounding the fungus is similar in appearance to the host cell wall (fibrillar) and since this area reacts positively to the PATAg (silver proteinate reaction) test for polysaccharides and to the Swift test for proteins, it is considered to be of host origin (Dexheimer et al., 1979; Scannerini and Bonfante-Fasolo, 1979; Bonfante-Fasolo et al., 1981). The material is thickest near the arbuscule trunk but still occurs around the finest arbuscule tips. The material begins to surround the arbuscule during the very early stages of infection and is termed the "apposition layer." The fact that it is thinner around the fine arbuscule tips indicates transfer facilitation. As arbuscule tips senesce and form aggregates, the apposition is substantially increased.

Cox and Sanders (1974) described multivesicular paramural bodies termed lomasomes in the small arbuscular hyphae. The vesicles associated with these lomasomes averaged 20 nm in diameter. Invagination of host plasmalemma resulted in paramural bodies that contained vesicles with an approximate diameter of 100 nm. Host produced paramural bodies were also

reported by Kariya and Toth (1981) and were observed to occur in the translucent portion of the interfacial matrix. It is possible that these bodies have a function in the transfer between the two organisms or aid in the formation of the interfacial matrix. Numerous changes in the host's cytoplasm, in addition to volume increases, accompany arbusculation. These include the occurrence of a highly polyploid host nuclei and a hyperactive golgi system (Scannerini and Bonfante-Fasolo, 1983). Host nuclei are observed to be in intimate contact with fungal proliferations (Carling and Brown, 1982).

Large numbers of mitochondria and protoplastids are observed in cytoplasm surrounding the enlarged nuclei. The inability of plastids to develop beyond the protoplastid phase and the general lack of starch grains in amyloplasts in infected cortical cells indicates an alteration of carbohydrate physiology. Concomitant with arbuscule senescence is the reversion of the host cell vacuole to its normal size along with a decrease in the numbers of mitochondria and other organelles. The decrease in fungal as well as host organelles during the very early stages of arbuscule senescence and the lack of observable polyphosphate in fungal tissue at this time supports the hypothesis that transfer between the two organisms occurs across living membranes before arbuscule senescence. Additional support for this hypothesis is the fact that the host plasmalemma does not

exhibit appreciable morphological or cytochemical modifications after the initial invagination in response to arbusculation, as is common with pathogenic infections. Specific alteration of the distribution of DES (diethylstilbestrol - a specific inhibitor of plasmalemma bound ATPases) - sensitive plasmalemma - bound ATPase activity is observed after the invagination of host plasmalemma following arbusculation (Marx et al., 1982). The ATPase activity, which was associated with the finest arbuscule tips, was not observed with very young or newly senescing arbuscules. This is, yet, another indication that active transfer processes are operating across living membranes and that the arbuscule is functional for a short time only.

The factors, or conditions, responsible for arbuscule formation are not understood. There is some evidence, however, in support of an elegant hypothesis that relates root exudation rates with infection levels. Ratnayake et al. (1978) found that the percentage of root P was correlated with the amount of P fertilization and that high levels of soil P, the exudation of soluble amino acids and reducing sugars was significantly reduced. Increased permeability of root membranes at low P was associated with lower phospholipid levels. Arbusculation would be encouraged in a cell low in P, due to the resulting lack of membrane integrity and subsequent high rates of exudation.

A recent report casts some doubt on this hypothesis, however (Schwab et al., 1983). The authors compared the stages of infection of Glomus mosseae in the roots of sudan grass. They found no differences in infection during the first twenty-five days of the study with P fertilization. External hyphae were more prolific in P deficient soil at twenty-five days, however. They concluded that the inhibition of external hyphae by high soil P reduced subsequent root penetration resulting in the much lower rates of root infection at high P observed at day thirty-five. It is possible that infection is related to internal and external effects of P.

Ultrastructural studies have resulted in the observation of several interesting inclusions in the VAM system. MacDonald et al. (1982) reported the occurrence of bacterium-like organelles (BLOs) in spore, hyphal and arbuscular protoplasm of VAM fungi. They mention that until they can be cultured, their bacterial nature is questionable. These entities, should they prove to be bacterial, could have significant implications for possible future genetic manipulations. The possibility that BLOs exist separately is discussed and intimates that the hyphae might take up such structures. Jabaji-Hare and Stobbs (1984) found no evidence of adsorption of virus particles by VAM hyphae in coinfected tomato roots. Tzean et al. (1983) observed helical organisms

that resembled spiroplasmas in hyperparasite infected VAM cytoplasm. Attempts to cultivate these cell wall free helical organisms were not successful.

Sugar maple is an important tree species that has not been given sufficient research attention to date, especially with regard to its mycorrhizal symbiosis. In fact, as foresters, we can hardly grow this complex species and rely primarily on natural regeneration in the field. It is apparent, after the above discussion, that mycorrhizal symbiosis is an integral part of a plant, affecting numerous aspects of its physiology. A great deal of our understanding of VAM symbiosis has resulted from research performed at the ultrastructural level. Anatomical investigations at the ultrastructural level on the VAM symbiosis of hardwood tree species have been few in number. Anatomical observations and determinations of the effect of the symbiosis on C and P physiology as well as on growth and development were combined in the present research in an attempt to understand the importance of the symbiosis to this species.

Explanation of thesis format

This thesis follows the alternate format outlined in the Graduate College Thesis Manual Handbook, Iowa State University, Ames, IA (Revised ed., 1981). Each part of the thesis represents a manuscript for publication with authorship and title as follows:

1) Yawney, W. J. and R. C. Schultz. 1985. Anatomy of a vesicular-arbuscular endomycorrhizal symbiosis between sugar maple and Glomus etunicatum.

2) Yawney, W. J., R. C. Schultz, T. H. Hilson, and C. W. Mize. 1985. The influence, over time, of inoculation, with the V-A endomycorrhizal fungus Glomus etunicatum, on growth, carbohydrate content and mineral nutrition of sugar maple seedlings grown at three soil phosphorus levels.

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SECTION I: ANATOMY OF A VESICULAR-ARBUSCULAR ENDOMYCORRHIZAL SYMBIOSIS BETWEEN SUGAR MAPLE AND *GLOMUS ETUNICATUM*

Anatomy of a vesicular-arbuscular endomycorrhizal symbiosis between sugar maple and *Glomus etunicatum*

W. J. Yawney
R. C. Schultz

The authors are, respectively, Graduate Student and Professor, Department of Forestry, Iowa State University, Ames, Iowa 50011. This paper is part of a Ph.D. Dissertation of the senior author.

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INTRODUCTION

Recent intensive research has established a general model that portrays vesicular-arbuscular endomycorrhizal (VAM) symbiosis as a highly dynamic physiological interaction. Nearly all aspects of a host's physiology are affected (Hayman, 1983).

The effect that has received the most attention is the improved phosphorus (P) nutrition of host plants relative to plants that lack the symbiosis in media deficient in available P (Mosse, 1973; Nicolson, 1967; Gerdemann, 1968; Harley, 1969). Infection by these fungi creates a reciprocal interaction where benefit to the host is accompanied by a photosynthate sink in the fungus (Harley, 1975; Cox et al., 1975; Losel and Cooper, 1979). The degree of intimacy expressed between the two organisms involved in a VAM symbiosis is so great as to preclude separation of discrete effects on the physiology of either. The site of intimacy between the host and endophyte is the arbuscule (Kinden and Brown, 1975a), which is formed in host cortical cells exclusively in the zone of differentiation and elongation in actively growing feeder roots. This dictates the dynamic character of the infection since the habitat continually changes location. The life span of an arbuscule has been estimated at from four to fifteen days (Bevege and Bowen, 1975; Cox and Tinker, 1976). The structure is a functional

exchange organ for only part of this time (Marx et al., 1982).

The sequence of events from arbuscule inception to senescence has been described in great detail (Cox and Sanders, 1974; Carling and Brown, 1982). Scannerini and Bonfante-Fasolo (1983) provided an excellent summary of current ultrastructural literature dealing with all aspects of the VAM system. The emphasis of the present paper will be the infection cycle.

Present ultrastructural investigations have been performed on a limited number of plant species and an even smaller number of tree species (Kinden and Brown, 1975a; Kinden and Brown, 1975b; Kinden and Brown, 1975c; Kinden and Brown, 1976; Strullu et al., 1981). Sugar maple (Acer saccharum Marsh) is one of the most important tree species in North America and has been known to form the symbiosis since 1914 (McDougall, 1914). Kessler (1966), using light microscopy, provided an anatomical description of VAM sugar maple roots, collected from the field. Infection in this species has not been described at the ultrastructural level, however. The present research investigated host and fungal changes during the cycle of infection using light, scanning electron and transmission electron microscopy.

METHODS

Sugar maple seedlings were grown from seed in 4-inch plastic pots in a steam sterilized turfas:sand (1:1 by volume) media in a greenhouse. Phosphorus was supplied by mixing rock phosphate (hydroxyapatite) with the media at a rate of 15 mg/kg. Other nutrients were supplied in solution on a biweekly basis. Nitrogen and potassium were provided by a commercial 15-0-15 fertilizer at the recommended rate. Magnesium and sulfur, Mn, Zn, Cu, Mo, and B were supplied by $MgSO_4$ (325 mg/l), $MnCl_2 \cdot 4H_2O$ (1.81 mg/l), $ZnSO_4 \cdot 7H_2O$ (0.22 mg/l), $CuSO_4 \cdot 5H_2O$ (0.08 mg/l), $(NH_4)_6Mo_7O_{24}$ (0.09 mg/l) and H_3BO_3 (2.86 mg/l), respectively. Iron was supplied in chelated form ($Na_2FeEDTA$) at a rate of 5 mg of Fe per liter. Due to high levels of Ca in the water supply, no additional Ca was required.

The species of VAM fungi selected for use in the study were Glomus etunicatum and Glomus mosseae. The fungi were originally isolated from central Iowa and maintained in pot culture on sorghum (Sorghum vulgare var. rorburghi) in a greenhouse. Spores were collected by subjecting the pot culture soil to a nematode illutriation technique (Sasser and Jenkins, 1960) and stored at 2° C in Ringer's solution. Inoculation consisted of adding 75 spores, in solution, to the bottom of a Watman number 42 filter paper cone buried in each pot. Nonmycorrhizal seedlings were treated identically

excluding inoculation.

Sugar maple seeds were collected from a registered, genetically superior individual in Essex Junction, Vermont in 1981 by researchers at the George D. Aiken Sugar Maple Research Laboratory, USDA, North Eastern Forest Experiment Station in Burlington, Vermont. Sound seeds were separated using a pentane flotation technique (Carl et al., 1969). Seeds were air dried to 10% moisture and frozen for storage (Yawney and Carl, 1974). Seeds were removed from refrigeration and allowed to equilibrate at 25° C for 24 hours, soaked in 5% sodium hypochlorite for 10 min and rinsed in 0.1% HCl followed by 8 rinses in tap water. Seeds were stratified at high moisture at 2° C (Carl et al., 1966) until germination was observed on day 37. Germinated seeds were planted directly above the buried filter paper cones at a rate of 3 per pot. After establishment, the seedlings were thinned to 1 per pot.

Seedlings were destructively sampled at 7, 14, 28, and 35 days, cleared and stained and examined for the presence of VAM infection. The roots were removed from soil by soaking the root mass in water. The roots were cleared and stained for the presence of VAM fungi (Kormanik et al., 1980). Samples were examined using a dissecting microscope and representative stages were recorded on a Zeiss photomicroscope.

Root systems were sampled and prepared for electron

microscopy at 35, 40, and 45 days. The first EM sample coincided with the initial appearance of infected cells in the root clearings in an attempt to record the early stages of the infection process.

Roots of sugar maple seedlings were gently removed from soil by soaking the root mass in 0.1 m phosphate buffer. Roots were then submerged in fixative which consisted of 3 percent glutaraldehyde and 1.5 percent paraformaldehyde in 0.1 m phosphate buffer at 4° C. The roots were sliced into 1 mm segments, placed in vials in fresh fixative and stored at 4° C for 6-10 hours. Fixative was removed with three 10-minute rinses in 0.1 m phosphate buffer and the specimens were post-fixed in 1% osmium tetroxide in phosphate buffer at 4° C for 2 hrs. Osmium was removed with three 10-minute, phosphate buffer rinses and the specimens were dehydrated in a graded ethanol series which consisted of 5 minutes each in 12, 25, 37, 50, 60, 70, 82, 95, 100, 100, and 100% ethanol.

Transmission Electron Microscopy

Specimens were then graded from 100% ethanol into propylene oxide with 10 minutes each in 3:1, 1:1, and 1:3 ethanol to propylene oxide, followed by 10 minutes each in 3 changes of 100% propylene oxide. Infiltration with Spurr's hard resin utilized an extended schedule as follows: 12 hours in 4:1 propylene oxide to resin; 24 hours in 3:2; 24 hours in 4:1 propylene oxide to resin; 24 hours in 100% resin. The

resin was cured at 60° C for 48 hours. Ultrathin sections were prepared with a Dupont Diamond knife on a Reichert Ultracut-E ultramicrotome and collected on 300 mesh copper grids. The sections were stained for minutes in methanolic uranyl acetate and 90 minutes in 2% lead citrate and observed with a Hitachi 11-C transmission electron microscope.

Light Microscopy

Thick sections (0.5μ - 1.0μ) were prepared with glass knives produced by an LKB microtome Knife Maker (Type 7801-A). The sections were placed on glass slides, stained with 1% toluidine blue at 95° C for 5 seconds and mounted in permount. Photomicroscopy was performed with a Zeiss photomicroscope.

Scanning Electron Microscopy

In 100% ethanol, specimens were carefully sliced in half with a clean, double edge razor blade in a wax coated petri dish while being observed at 10X on a dissecting microscope. Specimens were graded into toluene with 10 minutes in each 3:1, 1:1, and 1:3 ethanol to toluene followed by 10 minutes in 3 changes of 100% toluene. This solution was changed to 1:1 toluene to soluene for 24 hours at 22° C to remove host cytoplasm and reveal plant cell walls and fungal structures (King et al., 1981). Specimens were graded back into 100% ethanol and placed in 3:1 ethanol to freon 113 for 2 hours, 1:1 for 4 hours, 1:3 for 4 hours and 2 changes of

100% freon for 4 hours each. Specimens were placed in fresh freon, critical point dried with carbon dioxide, mounted on brass discs with silver paint and silver metal tape and coated with a gold-palladium mixture in a Polaron E5100 sputter coating unit. SEM observations were performed with a JEOL JSM-35 microscope at accelerating voltages of 15 to 25 kV with lens currents from 60 to 90 μA.

RESULTS

Infection was first detected in the cleared and stained root systems of destructively sub sampled seedlings at day 35. The infections observed at this time were limited to three or four widely scattered individual infection units per root system. An infection unit is used here to describe internal mycelium associated with a single root entry point (Wilson, 1984).

A single infection unit is characterized by two infection fronts that move longitudinally in the root in opposite directions from the entry point (Gallaud, 1905; Cox and Sanders, 1974). An infection unit, therefore, represents an age sequence with the oldest part of the infection near the entry point. Roots were prepared for light, scanning electron, and transmission electron microscopy (LM, SEM, TEM, respectively) at this stage in order to take advantage of the sequence apparent in these infection units. The authors have been able to detect infection units in sycamore roots in ethanol during the fixation process or in the polymerized resin. This was not the case with sugar maple, however, limiting the location of infection units to a search procedure that required extensive sectioning.

At day 45, infections were much more extensive, although, discrete infection segments could still be detected. An infection segment is defined as a discrete area of infection

that may arise from one or more entry points according to Wilson (1984). When individual units overlap, subsequent infection of a cell containing a senescing arbuscule may occur, resulting in more than one stage in a single cell. It is still possible, however, to find newly infected cells at the edges of infection segments.

Root segments from day 45 were heavily infected and contained numerous examples of arbuscule senescence as well as cells, that contained numerous stages due to multiple reinfection.

Sugar maple fine roots averaged 330 µm in diameter and contained cortical cells that ranged in size from 50 µm by 50 µm to 230 µm by 80 µm (Figs. 7 and 8). Roots of species from the genus Acer are characterized by a 'beaded' morphology that is thought to result from alternately favorable and unfavorable moisture conditions (Kessler, 1966)(Fig. 1). Kessler (1966) concluded that the beaded root morphology expressed by sugar maple is not the result of mycorrhizal infection as previously believed. Rather, it appeared to be related to alternately favorable and unfavorable soil moisture conditions since the phenomenon was not observed deep in the soil or in hollows but was restricted to surface layers and hummocks. Conditions in the greenhouse pots provided a wet-dry moisture cycle, although, not as extreme as one would expect to encounter in the natural system. As expected, less

beaded roots were observed in the study roots than are commonly observed in field collections (personal observation). The beaded appearance results from constrictions that occur at various places along the fine roots. The constrictions generally range from 100 to 500 μm. Cortical cells preceeding the constriction are reduced in size to approximately 20 μm by 10 μm, indicating that moisture may have indeed been limiting, thus reducing cell expansion. In addition, cortical cells appear to regain full size immediately following the constriction suggesting that ample moisture was again available for cell expansion. The beaded morphology did not have any apparent effect on infection since it commonly occurred near and on either side of the constriction. It was not determined, however, whether hyphae can pass through the constriction or if separate external entry is required on either side.

The nearly complete lack of cytoplasm and its location closely oppressed to the cell wall in uninfected control root cortical cells is evident at the light microscope level (Fig. 2). With inoculated roots, proliferations of external hyphae were observed on root surfaces at day 28, before internal infection was present. Infection began with the formation of an appressorium-like structure and subsequent penetration between or through epidermal cells (Fig. 3). Very few intercellular hyphae were observed at the LM, SEM, or TEM

level once root entry had occurred. Hyphae appeared to ramify the cortical zone by moving primarily intracellularly from cell to cell (Figs. 7 and 8). Intercellular and intracellular hyphae were similar in contents and appearance. Intracellular hyphae ranged in size from 2 to 5 μm in diameter. Intracellular hyphae were at times highly cytoplasmic and contained numerous organelles including mitochondria (0.3-0.4 μm), nuclei (2-3 μm), endoplasmic reticulum, lipid bodies and tonoplast bound electron dense bodies (0.2 - 0.6 μm) (Figs. 8 and 10). The latter have been shown by Cox et al. (1980), using x-ray microanalysis to contain polyphosphate. Large areas of cytoplasm were indistinct in appearance except for numerous small glycogen bodies. Other intracellular hyphae contained primarily irregularly shaped vacuoles that ranged in size from 0.4 to 1.5 μm (Figs. 11 and 12). Nuclei were apparent in these hyphae as well. Intermediate stages between the highly cytoplasmic and vacuolate hyphae were present.

Hyphal constrictions were observed at cell wall entry points (Fig. 5). No physical change to the cell wall was apparent with cell entry except for a possible slight disalignment of the wall pieces or respective sides of the hyphae (Figs. 5 and 6). Hyphae that penetrated cortical cells often passed through a cell without subsequent modification. Alternately, intracellular hyphae were observed to form coils

or arbuscules.

At the point of entry, a "collar" of material, similar in appearance to the host cell wall surrounded the invading hyphae (Figs. 5 and 6). This material was less osmiophyllic than the fungal wall it surrounded and appeared to have a random microfibril arrangement set in an unstained matrix. This agrees with descriptions reported by Cox and Sanders (1974) and Kariya and Toth (1981). Cell entry did not result in the disruption of the host plasmalemma in any case. Rather, the plasmalemma maintained its integrity during hyphal entry, proliferating to afford intimate contact with all intracellular fungal structures formed.

Vesicles were not observed in any of the study roots examined. Coiled intracellular hyphae were similar in content to uncoiled intracellular hyphae.

Arbuscules were the result of repeated dichotomous branching that occurred to such an extent in certain cases, that the entire cell column was occupied by short bifurcate hyphal branches that ranged in size from 0.4 μm to 0.8 μm (Figs. 15-19).

Arbuscules were always associated with large intracellular hyphae. When the cell environment in a cortical cell was suitable for arbuscule formation, several initiation points lead to the dichotomously branched proliferations in apparent synchrony. In other words, arbusculations in a young

arbuscule were at the same stage even when they arose from separate initiation points in different parts of the cell, as observed with the SEM.

Young arbuscule tips were densely cytoplasmic (Fig. 18) and became progressively vacuolate as they aged (Figs. 19-20). Eventually, the cytoplasm became disorganized and subsequently disintegrated. Collapse of the fungal wall followed (Figs. 19-20). The collapsed branches coalesced into dense aggregate clumps (Figs. 24-27). A complex remnant mass resulted. It was apparent, after observing several stages of arbuscule deterioration, that structures making up the mass were related to former arbuscle tips (Fig. 27). Larger masses at the same stage, therefore resulted from larger arbuscules. Masses decreased in size and became increasingly amorphous with age Fig. 9-11). This agreed quite well with descriptions presented by Kinden and Brown (1976).

The increase in plasmalemma surface area that accompanied cell invasion was greatest by far with arbuscular formation. A large increase in host cell cytoplasm with the formation of these fungal organs was also observed. Cox and Tinker (1976) analyzed tracings of electron micrographs of mycorrhizal onion roots with an image analyzing computer and calculated increases in host plasmalemma surface area and host cytoplasm volume of 3 and 23 fold, respectively. Visual examination of scanning electron micrographs of large arbuscules in the

present study suggest at least a 3-fold increase in plasmalemma surface area (Fig. 17).

Host cytoplasm, associated with young arbuscules, contained irregularly shaped, enlarged nuclei (15-20 μm), numerous mitochondria (0.4-0.8 μm), protoplastids (2-8 μm), endoplasmic reticulum and ribosomes and occasionally golgi bodies (Figs. 18-20). The host nucleus was displaced, from its usual position closely oppressed to the cell wall; to facilitate intimate contact with young and senescing arbuscule stages. A decrease in the number of mitochondria and other organelles was associated with deteriorating arbuscules (Fig. 26). As arbuscule tips continued to degenerate and collapse into a mass, it was as if they pulled out of the surrounding cytoplasm. The resulting collapsed mass was no longer associated with host cytoplasm which appeared to return to its normal volume.

The arbuscule branches and host plasmalemma were never in direct contact but were always separated by an interfacial region. Two distinct subregions were observed. The area on the fungal side of the region was occupied by a granular and (or) fibrillar electron dense matrix that was less osmiophyllic than the fungal wall. The plasmalemma side of the region was seen as a translucent space devoid of structure. However, the study did not use the simultaneous gluteraldehyde - osmium tetroxide fixation procedure used by

Carling et al. (1977) which preserved a granular matrix in this translucent zone.

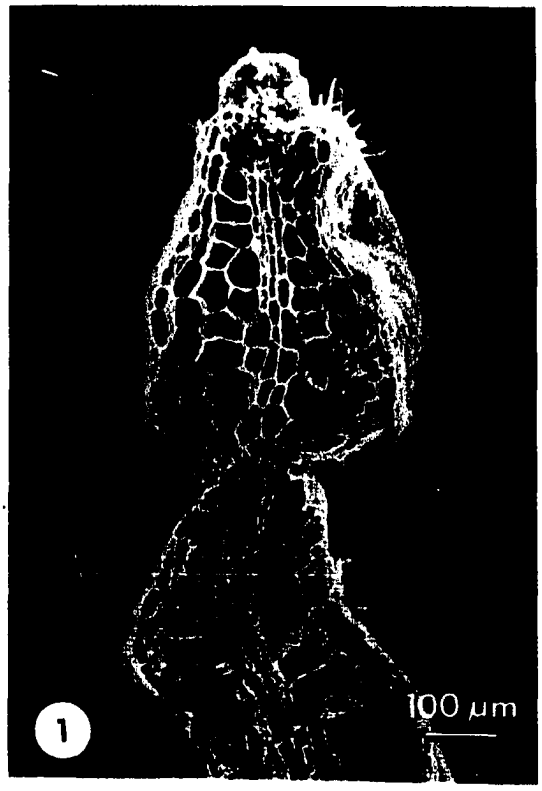
Deposition of material around fungal structures was greatest with larger diameter hyphae (Fig. 22). Deposition around the young, small diameter hyphal tips was, in fact, quite limited. As arbuscular hyphae senesced and ultimately collapsed, deposition increased substantially until a relatively thick, dense layer surrounded each collapsed hyphae. The deposition layer appeared to be of host origin due to its fibril appearance and level of osmium staining. Numerous discrete membrane bound vesicular bodies were observed in the interfacial region during arbuscule senescence. They averaged 60 nm in size and were generally associated with the translucent portion of the zone. Some of these bodies were empty, others contained material and some contained extremely small (4 nm) electron dense bodies (Fig. 21).

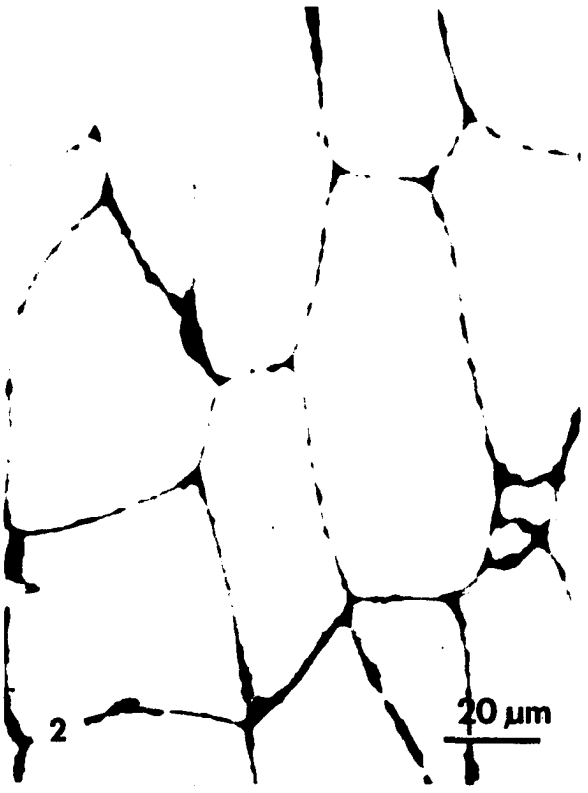
Figure 1. Scanning electron micrograph illustrating the characteristic morphology of a beaded root

Figure 2. Light micrograph of uninfected control root cortical cells

Figure 3. Light micrograph of a root clearing, illustrating the appressorium-like structures formed on root surfaces (A = appressorium)

Figure 4. Light micrograph of infected cortical region (N = nucleus; A = arbuscle; I = intracellular hyphae)


100 µm
1


2
20 µm


A
3
20 µm


A
N
I
4
20 µm

Figure 5. Transmission electron micrograph of hyphal entry, accompanying constriction (IH = interhyphal hyphae; C = collar)

Figure 6. Scanning electron micrograph of hyphal entry

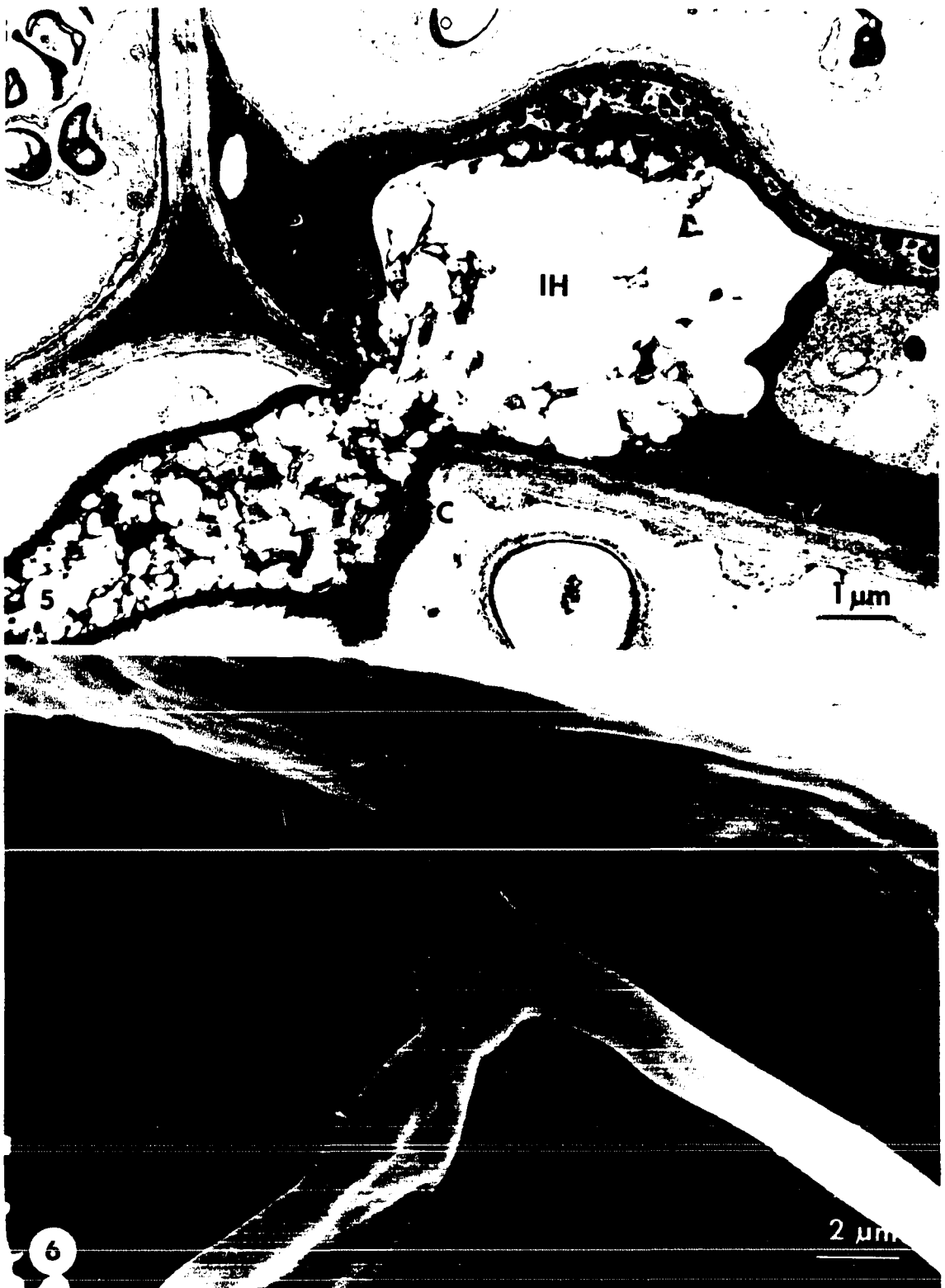
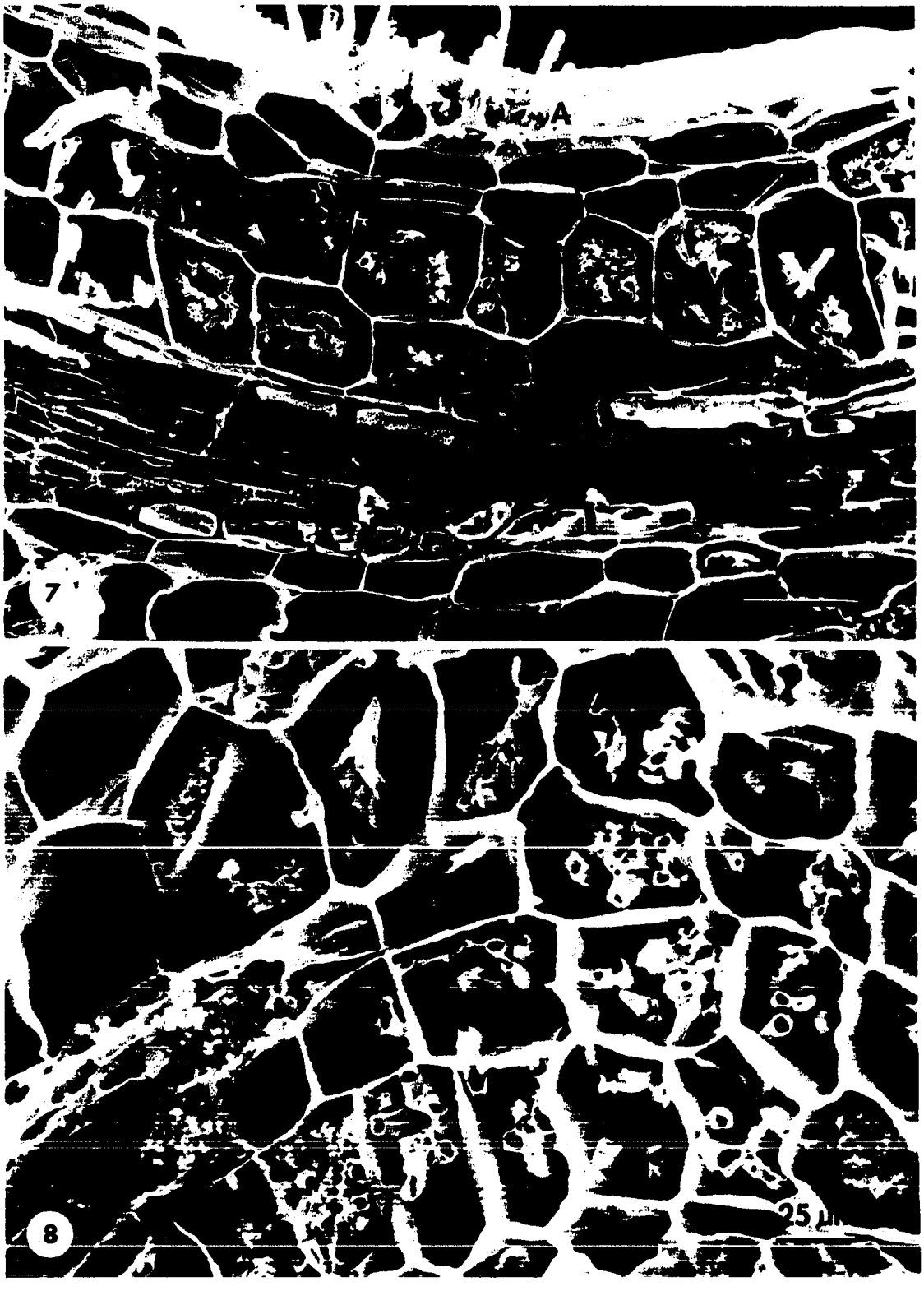

IH
C
5
1 µm
6
2 µm

Figure 7. Scanning electron micrograph of infected cortical region (A = arbuscule; C = coil)

Figure 8. Scanning electron micrograph of infected cortical region


A
7
8
25 µ

Figures 9 & 10. Transmission electron micrographs of densely cytoplasmic intracellular hyphae (N = fungal nucleus; EDB = membrane-bound electron dense body)

Figures 11 & 12. Transmission electron micrographs of vacuolate intracellular hyphae (HP = host plasmalemma; FP = fungal plasmalemma; FW = fungal wall)

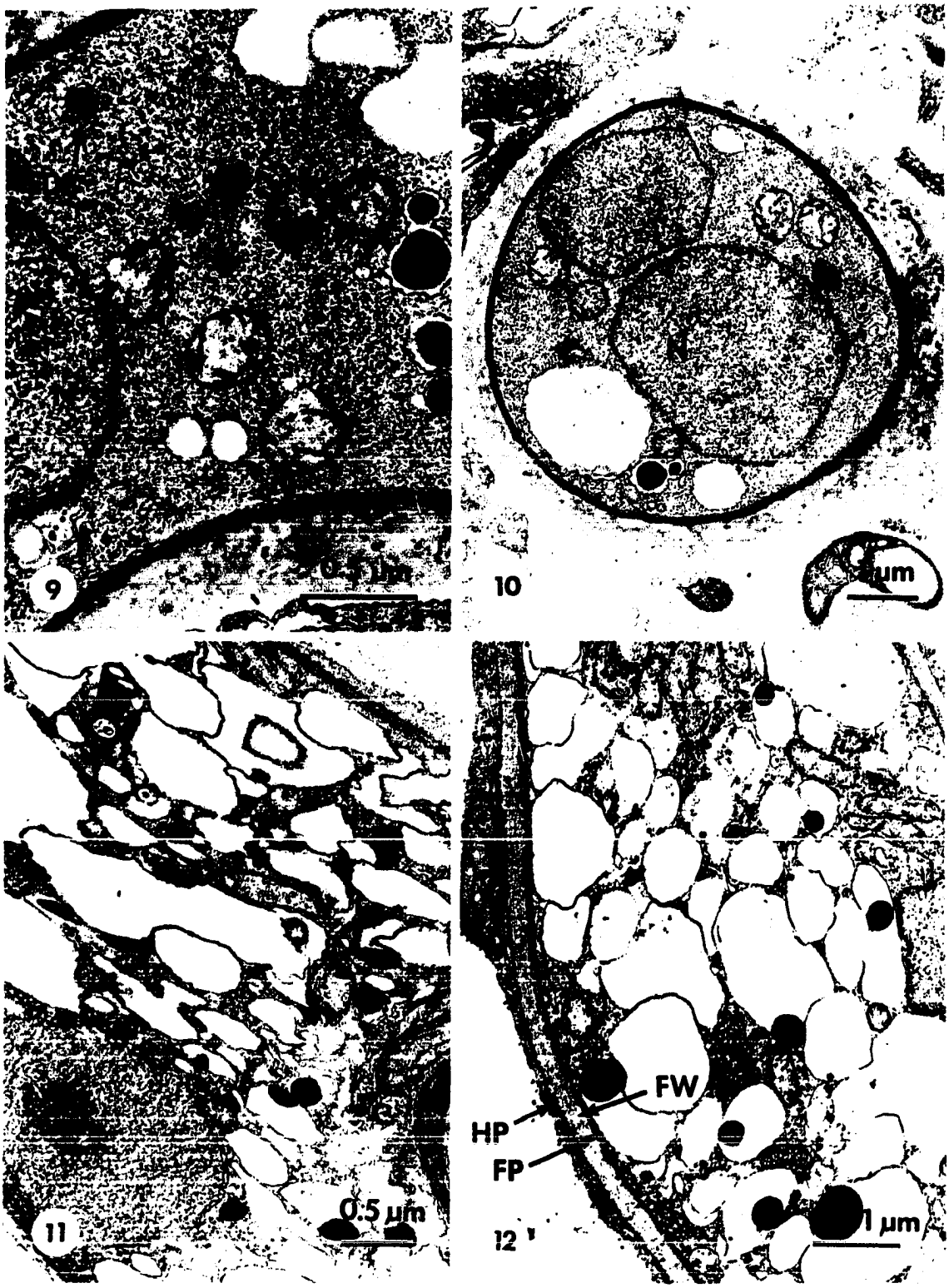

9
0.5 µm
10
µm
11
0.5 µm
FW
HP
FP
12
1 µm

Figure 13. Scanning electron micrograph of hyphal coils

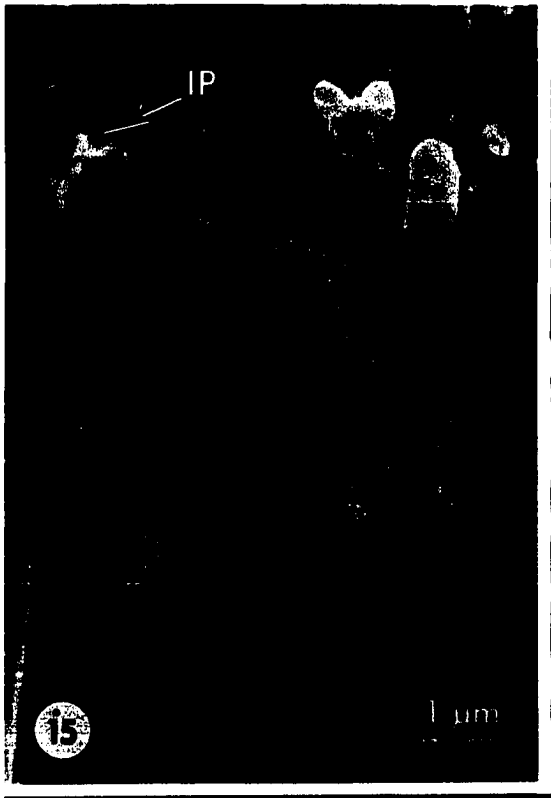
Figure 14. Scanning electron micrograph showing arbusculated coils


13
10
14

Figure 15. Scanning electron micrograph of arbuscule initiation point (IP = initiation point; H = large intracellular hyphae)

Figure 16. Scanning electron micrograph of a newly forming arbuscule

Figure 17. Transmission electron micrograph stereo pair illustrating the three dimensional surface area of the arbuscule


IP
1 µm
15


16

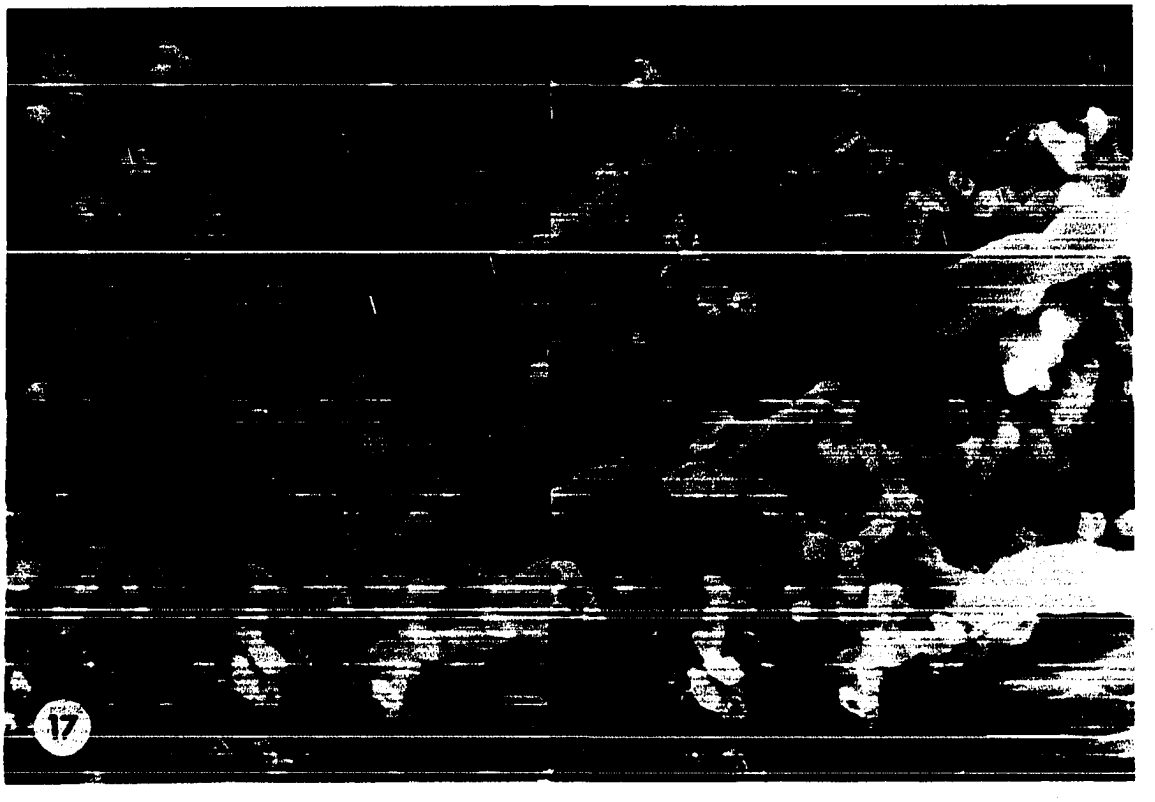
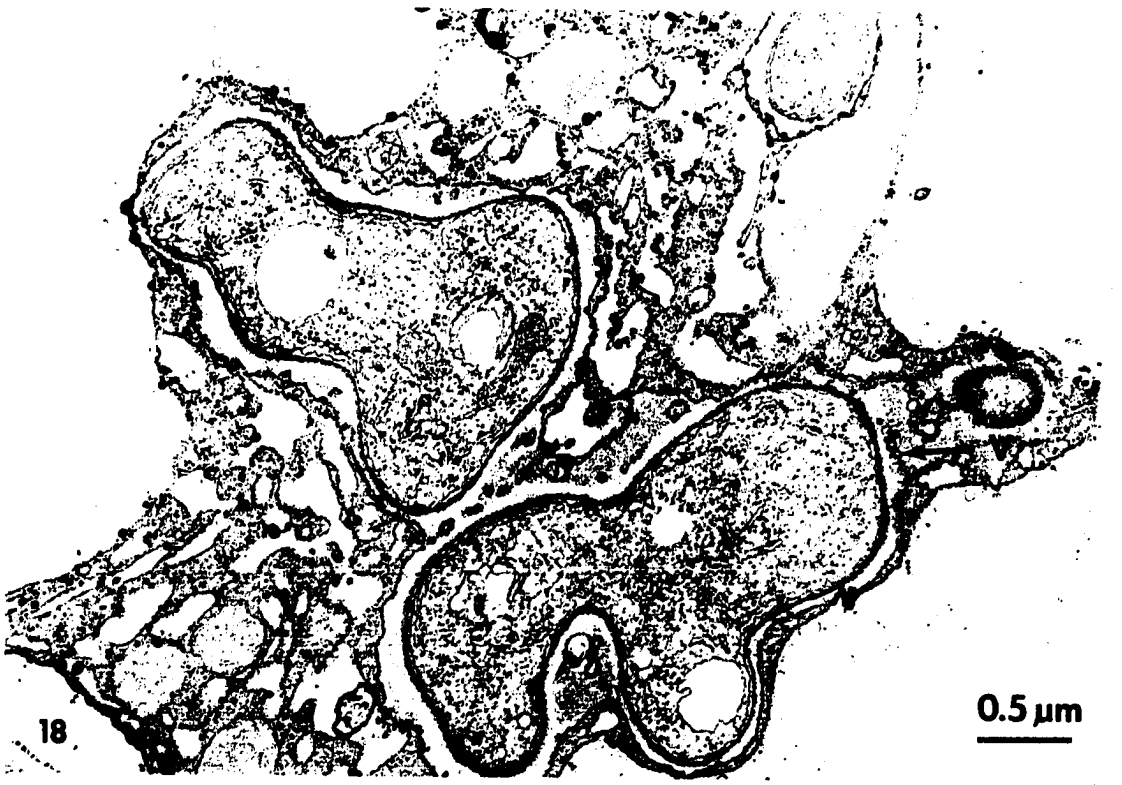

17

Figure 18. Transmission electron micrograph of young arbuscule tips in association with host cytoplasm (V = membrane-bound vesicular bodies)

Figure 19. Transmission electron micrograph illustrating arbuscle tips at various stages (A = young arbuscule tip; DA = degenerating arbuscule tip; CA = collapsed arbuscule tip)


18
0.5 µm


CA
19
1 µm

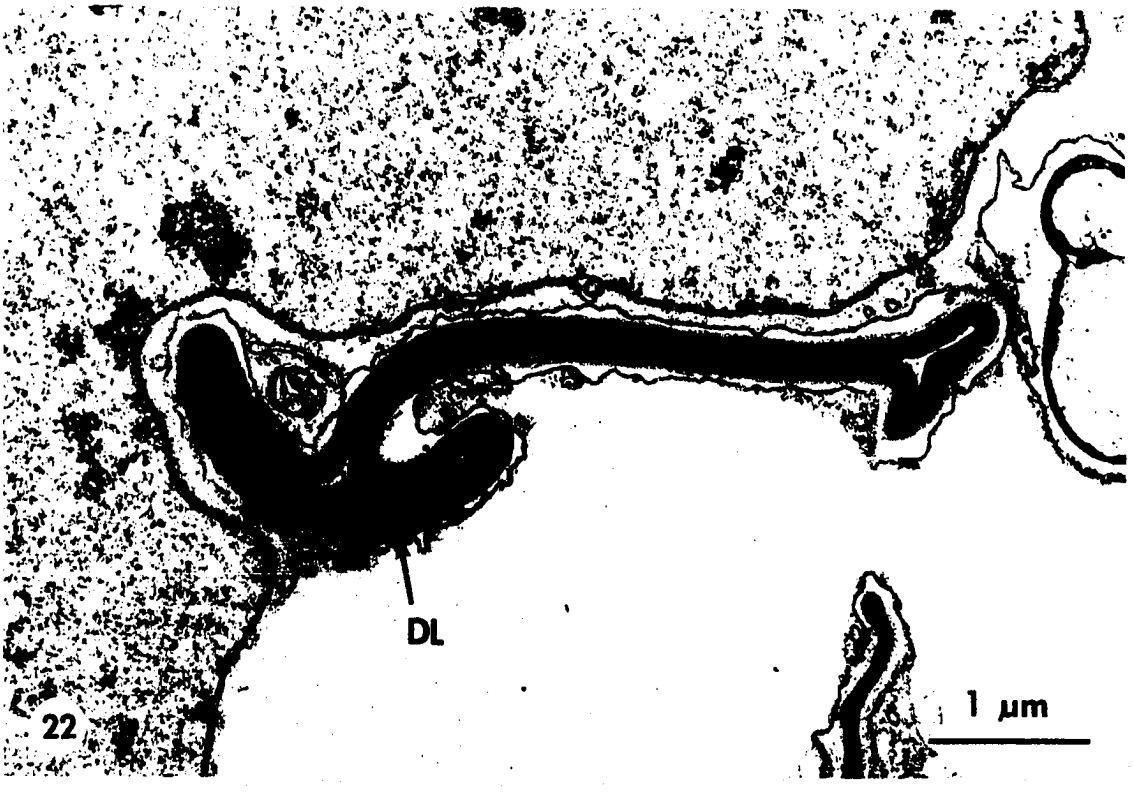
Figure 20. Transmission electron micrograph of vacuolating arbuscule tips (V = membrane-bound vesicular bodies; EDB = extremely small electron dense bodies)

Figure 21. Transmission electron micrograph of highly vacuolate arbuscular tips and degenerated arbuscule maps (N = host nucleus; RM = remnant mass)

2 µm
N
RM
21
2 µm
20
V
EDB

Figure 22. Transmission electron micrograph showing large intracellular hyphae after degeneration and its close association with the host nucleus (DL = deposition layer)

Figure 23. Transmission electron micrograph of lipid-filled arbuscule tips (L = lipid)


DL
1 µm
22

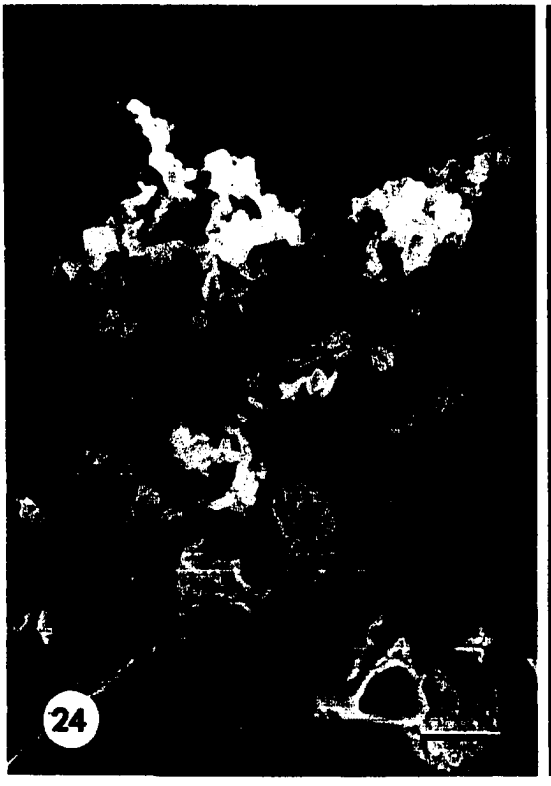

L
23

Figure 24. Scanning electron micrograph of a collapsing arbuscle

Figure 25. Scanning electron micrograph of a remnant mass that resulted from arbuscule degeneration

Figure 26. Transmission electron micrograph of collapsed intracellular hyphae that are still closely associated with the host's cytoplasm. Notice the numerous membrane-bound vesicular bodies present at this stage in the interfacial matrix

Figure 27. Transmission electron micrographh of a remnant mass



26

27

DISCUSSION

The lack of vesicle formation with the study host endophyte system is an interesting result. Vesicles are fungal storage organs (Cox et al., 1975) and it is possible that the production of host photosynthate was too low in the young seedlings to allow for their formation. However, vesicle formation in sugar maple roots was extremely rare in long term growth and development studies as well.

The pattern of arbuscular development observed in sugar maple roots, which consisted of arbuscular initiation at various places on large intracellular hyphae, differs from patterns described by other workers (Cox and Sanders, 1974; Kinden and Brown, 1975c; Cox and Tinker, 1976; White and Brown, 1979; Scannerini and Bonfante-Fasolo, 1983). These reports discuss the arbuscular trunk intimating immediate branching upon cell entry resulting in a terminate structure associated with that particular cortical cell. In the system discussed here, it appeared that hyphae entered and perhaps exited the cell with arbuscular formation occurring subsequently. It was as if hyphae randomly explored cortical cells forming arbuscules when the cell environment was suitable.

The factors that determine where these initiation points occur are not understood but may relate to localized differences in the host plasmalemma according to the

phosphorus (P) status theory presented by Ratnayake et al. (1978). Ratnayake et al. found that the percentage of root P was correlated with the amount of P fertilization and that at high levels of soil P, the exudation of soluble amino acids and reducing sugars was significantly reduced. Increasing permeability of root membranes at low P was associated with lower phospholipid levels. Arbusculation would be encouraged in a cell low in P due to the resulting lack of membrane integrity and subsequent high rates of exudation. It is possible that this phenomenon is not expressed uniformly throughout a single cell creating localized areas suitable for arbuscule initiation. This may also be due to membrane recognition factors.

The sequence of events observed, support the theory that the active arbuscule is the site of transfer between the host and endophyte (Scannerini and Bonfante-Fasolo, 1983). Evidence for this includes the fact that host and fungal organelles, necessary for active transfer processes, occurred simultaneously. This was true of their disappearance as well. In addition, the deposition of host cell wall-like materials, which could conceivably interfere with transfer, was quite low when the hyphae were cytoplasmic, but increased drastically as the hyphae became vacuolate.

Recent cytochemical evidence has supported the idea of active transfer at the living arbuscule. Nemec (1981)

demonstrated chitin in the walls and intercellular hyphae of VAM fungi but did not find it in arbuscular walls. Instead, the walls appeared to consist primarily of glycolipid. More specifically, the walls contained sphingolipids, which are known to function in membrane transport. Marx et al. (1982) found a specific alteration in the distribution of plasmalemma during arbuscule formation. The activity was associated with the finest arbuscule tips and was not observed with very young or newly senescing arbuscules, in other words, ATPase activity of the host and endophyte.

The apparent similarity of the apposition layer surrounding hyphal branches across the interfacial matrix to host cell wall material and the conclusion that it is of host origin is supported by cytochemical evidence. The substance reacts positively to the PATAg (Silver proteinate reaction) test for polysaccharides and to the Swift Test for proteins (Dexheimer et al., 1979; Scannerini and Bonfante-Fasolo, 1979; Bonfante-Fasolo et al., 1981). The occurrence of discrete membrane bound vesicular bodies in the interfacial region has been reported in other VAM systems (Cox and Sanders, 1974; Kariya and Toth, 1981). Their function in the transfer of materials between the two organisms or a possible role in the formation of the interfacial matrix has been observed. The observation in this study of extremely small electron dense

bodies in the vesicular bodies would tend to support the former.

CONCLUSIONS

The events observed and their sequences were very similar to those reported from other VAM plants. A unique factor involved in this work was the beaded root morphology expressed by sugar maple seedlings. This phenomenon appears to be related to periodic moisture stress as evidenced by the size of cortical cells formed prior to the constriction. The beaded morphology had no apparent effect on the infection process.

The occurrence of intercellular hyphae was limited, relative to other work and the pattern of arbuscule initiation was slightly different as well. Arbuscules were initiated at several places on large intracellular hyphae. This meant that few cortical cells contained exclusively terminate hyphal structures. Rather, hyphae that entered a cell, exited the cell as well. The living arbuscule appears to be the site of transfer between the host and endophyte and the process is an active one.

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SECTION II: THE INFLUENCE, OVER TIME, OF INOCULATION, WITH THE V-A ENDOMYCORRHIZAL FUNGUS *GLOMUS ETUNICATUM*, ON GROWTH, CARBOHYDRATE CONTENT AND MINERAL NUTRITION OF SUGAR MAPLE SEEDLINGS GROWN AT THREE SOIL PHOSPHORUS LEVELS

The influence, over time, of inoculation, with the V-A endomycorrhizal fungus Glomus etunicatum, on growth, carbohydrate content and mineral nutrition of sugar maple seedlings grown at three soil phosphorus levels

E. J. Yawney
R. C. Schultz
T. H. Hilson
C. W. Mize

The authors are, respectively, Graduate Student, Professor, Research Associate, and Assistant Professor, Department of Forestry, Iowa State University, Ames, Iowa 50011. This paper is part of a Ph.D. Dissertation of the senior author.

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INTRODUCTION

During the past thirty years, intensive research has established a general model that portrays VAM symbiosis as a highly dynamic physiological interaction. Nearly all aspects of the host physiology are affected (Hayman, 1983).

The effect that has received the most attention is the improved growth of plants with the symbiosis over nonmycorrhizal plants in media deficient in available phosphorus (P) (Mosse, 1973; Nicolson, 1967; Gerdemann, 1968; Harley, 1969). The P levels at which this phenomenon is expressed are quite common in the natural environment, as are these fungi, which are characterized as being ubiquitous.

Mycorrhizae is in a reciprocal interaction where benefit to the plant is accompanied by a carbohydrate drain on the host by the fungus (Harley, 1975; Cox et al., 1975; Losel and Cooper, 1979). Growth of the host is increased when benefits due to improved P nutrition outweigh this photosynthate drain (Bethlenfalvay, 1982b). Depressed growth of mycorrhizal plants, produced under conditions of high available P, relative to nonmycorrhizal plants is interpreted as expression of the carbohydrate drain (Buwalda and Goh, 1982; Yawney et al., 1982).

Mycorrhizal plants are often smaller and contain higher levels of P than P fertilized nonmycorrhizal plants indicating an inability of the mycorrhizal plant to fully express its

improved P nutrition due to the endophyte drain (Stribley et al., 1980). This is not always the case, however. Schultz et al. (1979) measured P concentrations in the larger mycorrhizal sweetgum seedlings that were half those found in nonmycorrhizal seedlings. This indicates the lack of a significant carbohydrate drain. In addition, Hayman (1983) feels that observed reductions in the growth of mycorrhizal plants relative to P fertilized controls may be too large to be accounted for by the carbon drain of an endophyte that comprises only about 10% of the weight of a mycorrhizal root system (Bevege et al., 1975; Hepper, 1977). The significance of a carbohydrate drain is, therefore, questionable and undoubtedly varies with the particular symbiont-host-environment interaction. Studies utilizing $^{14}CO_2$ indicate that the symbiosis has an effect on the distribution of host produced photosynthate (Snellgrove et al., 1982; Pang and Paul, 1980; Kucey and Paul, 1982). Such studies often find a greater amount of host photosynthate transferred to the below ground portion of mycorrhizal plants. A great deal of this photosynthate has been accounted for by greater below ground respiration associated with the symbiosis. It is important to note that the expression of improved P nutrition due to VAM symbiosis is highly dependent upon the N status of the growth media (Ames et al., 1983; Hepper, 1983; Hall et al., 1984).

Effect of VAM symbiosis on the growth and physiology of

host plants vary with plant age and environmental conditions or season. The sequence or progression of events that occur have been considered recently in a number of studies that have increased our understanding of this dynamic phenomenon (Bethlenfalvay et al., 1982c; Buwalda et al., 1982a; Buwalda et al., 1982b; Gay et al., 1982; Buwalda et al., 1984; Wilson, 1984).

It has been demonstrated that vesicular-arbuscular endomycorrhizal (VAM) symbiosis is a natural and integral part of the physiology of a wide variety of tree species (Fardelmann and McNabb, 1981; Guttay, 1982; Kormanik et al., 1976, 1977; Levy and Krikun, 1980; Pope et al., 1983; Riffle, 1980; Schultz et al., 1981; Verkade and Hamilton, 1983a, 1983b). Sugar maple (Acer saccharum Marsh) is one of the most important tree species in North America and has been known to form the symbiosis since 1914 (MacDougall, 1914).

Kessler (1966), using light microscopy, provided an anatomical description of VAM sugar maple roots collected from the field. However, research concerned with the growth and physiology of sugar maple (Taylor and Dumbroff, 1975; Dumbroff and Brown, 1976; Webb, 1976) have not considered the importance of the symbiosis to this species. Sugar maple is a very complex species that is not well understood but is considered to require high nutrition. Attempts to establish sugar maple artificially have generally met with failure

(Yawney, 1968). In view of this and considering the importance of VAM symbiosis to other plant species, it is apparent that the mycorrhizal factor should be investigated. The objectives of the present research were to determine the effect of mycorrhizal infection on the growth; carbohydrate content and mineral nutrition of sugar maple seedlings grown at three soil P levels as well as the distribution of these factors among plant parts over a one-year period.

METHODS

The study growth medium was comprised of peatmoss, perlite, and soil in a ratio of 1:1:1 by volume (Ward, 1980). Soil for the medium was a Dundas series, grey silt loam obtained under a red pine plantation at Holst Tract State Forest near Boone, Iowa. The particular soil was chosen because it was one of the few central Iowa soils tested that contained the desired low level of extractable phosphorus (P).

The soil was sieved through a 1.3 cm mesh screen to remove debris. Soil amendments were blended with equal volumes of peatmoss, perlite, and soil in a cement mixer for 20 minutes. Amendments consisted of a commercial 10•10•10 fertilizer at a rate of 280 kg/ha and 3 rates of dicalcium phosphate (0, 100, and 270 kg/ha) designed to adjust extractable P to the desired treatment levels of 35, 75, and 150. The media was enclosed in plastic and fumigated with methyl bromide at a rate of 1 can per 10 m^2 of soil 15 cm deep. This rate has been found to effectively eliminate pathogens or natural mycorrhizal inoculum in soils collected from the field (Menge et al., 1978; Riffle, 1980). Six liter, black plastic pots were filled with media, wrapped with aluminum foil to reduce the absorption of radiation and placed in their designated treatment positions in an outdoor lath house at the Iowa Conservation Commission State Nursery in Ames, Iowa.

The species of vesicular-arbuscular endomycorrhizal (VAM) fungus used in the study was Glomus etunicatum originally isolated from central Iowa. Inoculation consisted of mixing 200 spores with the media of each pot. The inoculum was produced in a greenhouse in pots on sorghum (Sorghum vulgare var. rorburghi). Spores were collected by subjecting the soil to a nematode illutriation technique (Sasser and Jenkins, 1960).

Sugar maple seeds were collected from a registered, genetically superior individual in Essex Junction, Vermont in 1981 by researchers at the George D. Aiken Sugar Maple Research Laboratory, USDA, North Eastern Forest Experiment Station in Burlington, Vermont. Sound seeds were separated using a pentane flotation technique (Carl and Yawney, 1969). Seeds were air dried to 10% moisture and frozen for storage (Yawney and Carl, 1974). The seeds were removed from refrigeration and allowed to reach thermal equilibrium at 25° C. The seeds were soaked in tap water at 25° C for 24 hours, soaked in 5% sodium hypochlorite for 10 minutes and rinsed 3 times in 0.1% HCl followed by 8 rinses in tap water. Seeds were stratified at high moisture at 2° C (Carl and Yawney, 1966) until germination was observed on day 35. Germinated seeds were planted June 3, 1982 at a rate of 6 per pot. After establishment, the seedlings were thinned to 1 per pot.

The seedlings received 50% shade provided by snow fence

on the top of the lath house. Moisture was supplied through nursery irrigation and was applied by hand when necessary. Supplemental nitrogen and potassium were supplied in the form of ammonium nitrate and potassium nitrate, respectively, on August 24, 1982 and March 21, April 15, and May 10, 1983 for a total of 500 kg/ha of N and 150 kg/ha of K. On November 25, 1982, after the seedlings had become dormant, they were placed in a barn to prevent rodent damage. The seedlings were returned to the lath house March 21, 1983.

A portion of the seedlings were destructively sampled on August 16, September 5, October 10, November 1, and November 15, 1982 and January 31, March 21, and June 2, 1983. Sampled seedlings were measured for height and stem diameter. Seedling leaf color was determined using the Munsell color system for plant tissue. Soil samples were taken from the pot of each sampled seedling and were analyzed for pH and extractable P, K, Ca, Mg, Fe, and Zn. Soil pH was determined using the 1:1 soil to water method of Peech (1965). The Bray I technique was used to determine extractable soil nutrients. Extractable soil P was detected with a Technican Auto Analyzer II (Industrial Method No. 94-70W, Technician Instruments Corp., Tarrytown, NY) and extractable K, Ca, Mg, Fe, and Zn were analyzed on a Perkin-Elmer Model 272 atomic absorption Spectrophotometer (Agricultural Method AY-2, Perkin Elmer Corp., Norwalk, CT).

Seedlings were removed from the pots by gently washing the soil from the roots. Samples of the fine feeder roots were taken from five places on the root system of each seedling and were stored in formalin acetic alcohol (FAA) for subsequent analysis of mycorrhizal infection. Leaves, stems, and roots were separated, placed in bags, frozen and freeze dried. Feeder roots were separated from structural roots by hand and dry weights were recorded for leaves, stems, structural roots, and feeder roots. Plant parts were analyzed for total carbohydrate and levels of N, P, K, Ca, Mg, Fe, and Zn. Carbohydrate levels were determined with enzymatic hydrolysis technique as described by Haissig and Dickson (1979). Freeze dried 50 mg samples were extracted three times with methanol:chloroform:water (12:5:3 v/v/v) to remove reducing sugars, phenolics and other soluables. The samples were hydrolyzed for 48 hours in 1.5 ml amylogucosidase enzyme solution per sample. The reaction was stopped when 0.15 ml aliquots of sample supernatants were diluted with 1 ml DNS (3,5-dinitrosalicylic acid) solution. The samples were then subjected to a hot water bath (70 to 100 °C) for 5 minutes to develop color and were cooled to room temperature. Subsequent colorimetric assay was performed at 540 nm on a spectrophotometer. Nutrient levels were detected by digesting plant parts in a solution of H_2SO_4-Se and H_2O_2 at 400° C for 110 min. (Technicon Industrial Methodology AAII, 98-70W).

Nitrogen (Kjeldahl) and phosphorus were determined using a Technicon Autoanalyzer II (Technicon Industrial Methodology AAII, 327-74W). Plant K, Ca, Mg, Fe, and Zn were determined using a Perkin-Elmer Model 272 Atomic Absorption Spectrophotometer.

VAM assay was made using the root clearing and staining schedule reported by Kormanik et al. (1980). The samples were evaluated for the percentage of roots infected and for the intensity of infection. This examination was performed under a dissecting microscope equipped with transmitted light. Each sample was placed into one of five infection classes: (i) 0 to 5% of the fine feeder roots showed some infection; (ii) 6 to 25%; (iii) 26 to 50%; (iv) 51 to 75%; (v) 76 to 100%. Intensity of infection was determined using the grid intersect method (Biermann and Linderman, 1981; Giovannetti and Mosse, 1980) and was based on 100 intersections, of a 1/2 cm grid, per sample. Particular care, which included the use of a compound microscope, was taken with samples from noninoculated seedlings to insure that they were free from mycorrhizal infection.

The study was a factorial (2 mycorrhizal conditions x 3 soil P levels x 8 sampling dates) experiment arranged in a randomized block split-plot design. Mycorrhizal and nonmycorrhizal seedlings were arranged in physically separated split plots within whole plots or blocks to avoid

contamination resulting from splashing from rain or watering. Phosphorus level by sampling date treatments were completely randomized in each split plot. There were 48 treatments with 7 blocks or replications per treatment for a total of 336 pots.

Data were analyzed using the Statistical Analysis System (SAS) (Barr et al., 1976).

RESULTS AND DISCUSSION

Initial seedling establishment was characterized by the formation of two primary leaves and a distinct terminal bud, and occurred 2-3 weeks after planting the pregerminated seeds. In those seedlings that grew beyond this stage, there was a delay of one to several weeks before they broke bud, indicating that root growth may have been favored temporarily.

At harvest 1, there were no apparent differences in seedling growth related to study treatments. Young expanding leaves, for seedlings grown at low soil P (LP), were red in color (5R-4/8 according to the Muncell Color System for plant tissue) and turned green (5 GY-6/8 to 5/8) darkened by reddish purple areas (5R-3/6 and 3/4) as they matured. Seedlings produced at medium and high soil P (MP and HP, respectively) were lighter red in color (5R-5/10 or 6/10), initially, than the leaves of LP seedlings at the same stage and had mature leaves that were a brighter, more uniform green (5GY-6/8).

By harvest 2, differences in seedling height and diameter, with treatment, were evident. There was a noticeable difference in color between inoculated and uninoculated seedlings at LP. Uninoculated seedling leaves had more extensive purpling and necrosis. Inoculated seedling leaves showed a little purpling in young leaves that changed to dark green (7.5 GY-4/4 to 4/6) when they became fully formed. At harvest 2, MP seedlings were clearly larger than

HP seedlings. Seedlings grown at MP and HP were characterized by leaves that were uniform in color (7.5 GY-4/4 to 5/6).

At harvest 3, seedling size differences with soil P level were readily apparent. The only indication of approaching dormancy was a slightly lighter leaf color overall, which may have indicated a breakdown of materials and mobilization out of the leaves. Differences in leaf color between inoculated and uninoculated seedlings at LP were the most pronounced at harvest 3. Uninoculated seedling leaves ranged in color from dark green-purple (2.5 G 4/4 to 3/4; 5R 3/2 to 3/4) in the center of the leaves to red (5R 3/6) and yellow (2.5 Y 6/8 to 5/6) between the major veins followed by necrosis. The necrosis occurred in spots that coalesced to form areas and progressed inward from the edges, between the veins. This is typical of the P deficiency symptoms that occur in sugar maple (Erdmann, et al., 1979). Inoculated seedlings at LP were similar in leaf color to those at harvest 2.

At harvest 4, nearly all seedlings had set terminal winter buds and had lost all or most of their leaves. The soil, in the pots, was frozen for extended periods between harvests 5 and 7.

Terminal dieback, that was usually confined to the upper node, was detected in 17 of 42 seedlings sampled at harvest 8. The occurrence of dieback was not related to study treatments. Low P seedlings, again, were characterized by visual P

deficiency symptoms. No difference was evident between inoculated and uninoculated seedlings, however. Seedlings in the MP and HP treatments had uniformly green leaves of a somewhat lighter shade (7.5 GY - 6/10) than those of the previous season.

Mycorrhizal inoculation, although responsible for significant levels of infection, had no detectable effect on seedling size but did influence root to shoot ratios. Mycorrhizal inoculation also had an effect on root carbohydrate percent and therefore whole plant carbohydrate percent as well as the levels of certain elements in plant parts. Harvest date and phosphorus fertilization rate influenced nearly all dependent variables observed during the study. The rate of phosphorus fertilization had highly significant effects on soil P, K, Ca, Mg, Fe, and Zn. These differences in the levels of soil elements influenced plant growth and the levels of certain elements in plant parts. Fluctuations in certain size parameters and the levels of certain elements followed the general pattern evident in Figure 1. Interactions of the main effects were common, but could be explained in most cases, allowing a clear identification of trends due to main effects.

The largest seedlings in terms of height, diameter and total dry weight were produced at MP (148 mg/kg) or 100 kg/ha of applied dicalcium phosphate (DCP) regardless of harvest

date (Table 1 and Figure 1). Seedlings grown at HP (193 mg/kg) or 250 kg/ha of DCP were significantly smaller than MP seedlings while the smallest study seedlings were produced at LP (33 mg/kg) or no added DCP.

Seedling height varied significantly with harvest date ($P = 0.0001$), soil P level ($P = 0.0001$) and the interaction of these study treatments ($P = 0.0007$). The tallest seedlings, with an average height of 22.9 cm at harvest 8, were grown at MP. Seedlings grown at HP obtained an average height of 17.0 cm at harvest 8 while those at LP averaged only 10.5 cm. Seedlings produced at MP and HP increased in height during the first season until bud set and leaf fall, which occurred between harvests 3 and 4 (October 10 and November 1, 1982, respectively). A slight decrease in average heights for these two soil treatments, over the winter, is attributed to the terminal dieback that occurred on a number of the study seedlings. A rapid increase in height from harvest 7 (March 21, 1983) to the maximum values obtained at harvest 8 (June 2, 1983) was observed for MP and HP seedlings. After germination, LP seedlings established 2-6 leaves and set bud with no further height growth observed during either growing season. It is possible that a threshold level of P necessary for terminal growth was greater than the average extractable level of 33 mg/kg present in the LP treatment.

Stem diameter was also influenced by soil P level ($P =$

Table 1. Mean seedling size (HT = height; DIA = diameter; TWT = whole plant dry weight) over time (harvest dates 1-8 = Aug, 16; Sept, 5; Oct, 10; Nov, 1; Nov, 15, 1982 and Jan, 31; Mar, 21; June, 2, 1983, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 medium P; 3 = high P)

		Harvest Date								
Treatment		1	2	3	4	5	6	7	8	SEM[a]
HT (cm)	1	10.1	9.6	9.2	9.7	10.3	10.8	11.0	10.5	
	2	12.1	14.8	13.0	14.7	15.7	15.4	13.7	22.9	1.3
	3	9.1	11.3	13.8	12.5	14.2	12.3	11.9	17.0	
DIA (mm)	1	1.9	2.3	2.5	2.5	2.8	2.7	2.7	2.8	
	2	1.9	2.9	3.6	3.8	3.9	3.5	3.6	4.1	0.3
	3	1.9	2.6	3.3	3.5	3.7	3.2	3.2	3.8	
TWT (g)	1	0.5	0.9	1.3	0.8	0.7	0.9	0.7	1.1	
	2	0.6	1.7	3.1	2.6	2.4	2.1	1.9	3.6	0.4
	3	0.6	1.3	2.6	1.9	1.8	1.5	1.2	2.5	

[a]SEM = standard error for the difference between 2 means.

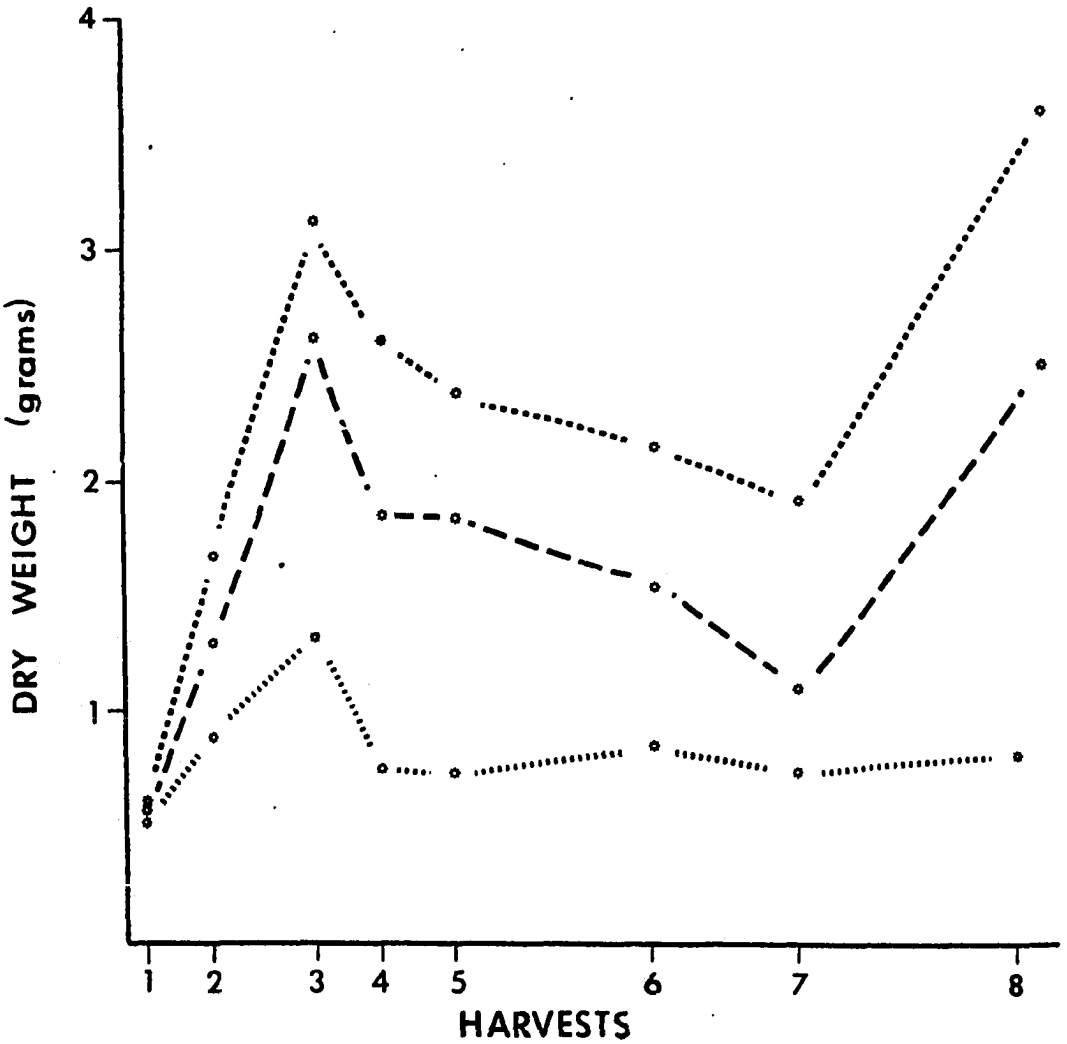

Figure 1. Mean whole plant weight over time (harvest dates 1-8 = Aug. 16; Sept. 5; Oct. 10; Nov. 1; Nov. 15, 1982 and Jan. 31; Mar. 21; June 2, 1983, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at three soil phosphorus (P) levels (····· = low P; ····· = medium P; - — - = high P)

0.0001) and harvest date (P = 0.0001) as well as their interaction (P = 0.0018). Stem diameters increased to maximum values of 4.1 and 3.8 mm at harvest 8 for MP and HP seedlings, respectively, but increased only to harvest 5 for LP seedlings where a maximum average value of 2.82 mm was recorded. The significant P level by harvest date interaction for stem diameter was due to the fact that diameter growth rates diverged from a common starting point at time zero. This interaction disappeared upon reanalysis excluding harvests 1 and 2 (P = 0.75). Divergent growth over time accounted for several other interactions as well.

Soil P level and harvest date and their interaction had significant (P = 0.0001 and 0.0001, respectively) effects on whole plant dry weights. The patterns of response were similar to those for height and diameter resulting in final whole plant dry weights of 3.6, 2.5, and 1.1 g for MP, HP, and LP seedlings, respectively. This was the maximum value for HP seedlings only. Low P and MP seedlings reached maximum dry weights of 1.3 and 2.6 g at harvest 3. The loss of dry weight from harvest 3 to harvest 7 is quite striking. It occurred rapidly between harvests 3 and 4, in association with leaf drop for all 3 P levels. A decrease in dry weight continued for MP and HP seedlings, although, not as rapidly until harvest 6, where it again decreased rapidly to harvest 7 for all 3 P levels. These decreases in dry weight reflect the

loss of leaves, the death of a significant portion of seedling feeder roots, that was observed visually during harvesting, terminal dieback and the use of carbohydrate reserves. The latter will be discussed below.

Inoculated seedlings had a lower average root to shoot ratio ($P = 0.0006$) than uninoculated seedlings (1.60 and 1.92, respectively). The effect of inoculation was not detectable at harvests 1, 6, and 7 accounting for a significant ($P = 0.0003$) harvest date by mycorrhizal inoculation interaction (Table 2). Dry weight distribution varied with harvest date ($P = 0.0001$) increasing rapidly from 0.26 at harvest 1 to 0.90 at harvest 3. Height growth was rapid up through harvest 2 but leveled off with an apparent switch in emphasis to root growth. The ratio was 3.3 at harvest 4, without the leaves, and decreased with feeder root death and use of carbohydrate reserves to 2.9 by harvest 7. The ratio at harvest 8, with full leaves, was 0.62. The dry weights of plant parts varied in response to study treatments in a manner similar to whole plant dry weight (Table 2). Leaf and stem maximum dry weights were reached at harvest 8 ($P = 0.0001$ and 0.0001, respectively) while structural root and feeder root weights were at a maximum at harvest 4. The inability of the seedlings to equal fall root weights, even with spring root production, can be attributed to substantial weight loss during the winter due to feeder root death and carbohydrate

Table 2. Mean dry weights for leaves (LW), stems (SW), structural roots (RW) and feeder roots (FRW) and root to shoot ratio (RTSR - bottom weight/top weight) in grams, over time (harvest dates 1-8 = Aug, 16; Sept, 5; Oct, 10; Nov, 1; Nov, 15, 1982 and Jan, 31; March 2 21; June, 2; 1983, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 = medium P; 3 = high P;) and either inoculated (I) or uninoculated (UI) with the vesicular-arbuscular endomycorrhizal fungus Glomus etunicatum

		Harvest Date								
	Treatment	1	2	3	4	5	6	7	8	SEM[a]
	1	0.34	0.46	0.48	-[b]	-	-	-	0.47	
LW	2	0.41	1.04	1.18	-	-	-	-	1.50	0.16
	3	0.34	0.74	0.96	-	-	-	-	1.02	
	1	0.07	0.14	0.21	0.20	0.20	0.23	0.22	0.20	
SW	2	0.08	0.24	0.47	0.59	0.55	0.55	0.46	0.72	0.07
	3	0.06	0.19	0.39	0.41	0.46	0.40	0.30	0.49	
	1	0.07	0.22	0.49	0.42	0.41	0.49	0.39	0.27	
RW	2	0.07	0.27	1.04	1.31	1.22	1.51	1.06	0.88	0.15
	3	0.07	0.23	0.86	1.01	0.93	0.78	0.66	0.69	
	1	0.03	0.07	0.14	0.14	0.13	0.14	0.13	0.12	
FRW	2	0.05	0.18	0.45	0.71	0.60	0.44	0.40	0.52	0.08
	3	0.05	0.13	0.42	0.44	0.43	0.36	0.24	0.30	
	1	0.25	0.47	0.90	2.79	2.79	2.78	2.39	0.57	
RTSR	2	0.23	0.31	0.90	3.46	3.31	3.54	3.18	0.63	0.18
	3	0.29	0.44	0.92	3.53	2.93	2.86	3.18	0.66	
	I	0.26	0.38	0.84	3.06	2.46	2.57	2.86	0.65	0.14
RTSR	UI	0.25	0.41	1.00	3.73	3.46	2.98	2.76	0.61	

[a] SEM = standard error for the difference between 2 means.

[b] No leaves due to dormant season.

depletion. Even though the weight was less at harvest 8, the root volume could have been greater because at harvest 4 the roots contained a much greater percentage of carbohydrate (discussed below).

The lack of an effect of mycorrhizal inoculation on seedling size parameters at low P is a significant result when considering the importance of the symbiosis to the growth of other hardwood tree species at low P such as box elder, black cherry, black locust, white ash, green ash, sweetgum, sycamore, walnut, and yellow poplar at (Kormanik et al., 1976; Kormanik et al., 1977; Schultz et al., 1979; Fardelmann and McNabb, 1981; Kormanik et al., 1981; Schultz et al., 1981). A low dependency on the symbiosis by this species or seed source (Crush, 1974; Baylis, 1972; Johnson, 1976; St. John, 1980), or, the use of a fungal species or isolate inappropriate for the study plants or study conditions (Carling and Brown, 1980; Graham et al., 1982) would explain this. Even though a growth response was absent at low P, visual and chemical (discussed below) evidence indicated that P nutrition was improved by inoculation. The fact that this improved nutrition was not translated into growth might have resulted from a lack of photosynthate for synthetic processes due to the carbohydrate drain on the host by the endophyte (Ho and Trappe, 1973; Cox et al., 1975). Mycorrhizal systems have been shown, with the detection of evolved $^{14}CO_2$, to have higher rates of

respiration in certain host-endophyte-environment systems (Pang and Paul, 1980; Kucey and Paul, 1982). The lower root to shoot ratio for inoculated seedlings may have reflected less need for large root systems due to the extensive exploitation of the soil by mycorrhizal hyphae (Tisdale and Oades, 1979), or, may be evidence of higher rates of respiration in inoculated roots, resulting in less use of below ground photosynthate for synthetic processes.

The effects of the independent variables soil P level and harvest date on seedling growth are clear. The reduction in growth observed at HP relative to MP may indicate a direct inhibitory effect of high soil P, or an indirect one expressed through an effect on the levels of other soil elements. An indication of a direct inhibitory effect of high soil P on the growth and establishment of sycamore seedlings in fumigated nursery soil has been observed (Yawney et al., unpublished data). The lack of height growth in seedlings at low P, even with improved P nutrition associated with inoculation could have resulted from a lack of carbohydrate in the inoculated situation as discussed or may indicate the existence of a threshold level of P that must be surpassed before sugar maple will invest in height growth.

Carbohydrate percent of the whole seedling varied significantly with P level ($P = 0.0001$), harvest date ($P = 0.0001$) and mycorrhizal inoculation ($P = 0.028$) as seen in

Table 3 and Figure 2. Whole plant carbohydrate percent increased rapidly from 6.2% to 22.4% during the period between harvests 1 and 3. A less rapid, yet steady decrease, followed resulting in a level of 5.2% by harvest 8. The highest levels of carbohydrate during the first growing season were in the LP seedlings. This could have been due to the lack of growth exhibited by these seedlings and a subsequent lack of need for produced carbohydrates. There was very little difference between seedlings grown at MP and HP regardless of harvest. There was an indication ($P = 0.06$) of a mycorrhizal effect on whole plant carbohydrate percent. Uninoculated seedlings had a greater percentage of carbohydrate for harvests 3-7. The largest difference of 6.3% occurred at harvest 5 and was the result of a relatively rapid decrease in the carbohydrate percent of inoculated seedlings between harvests 4 and 5. It is believed, that lower levels in inoculated seedlings is evidence of the carbohydrate drain of the fungus on the plant. The rapid decrease in mycorrhizal seedlings during the late fall (harvests 4-5) is thought to be due, at least in part, to a rapid storage of carbohydrate by the fungus in soil hyphae and spores as preparation for winter survival.

Carbohydrate was partitioned differently among plant parts with very low levels in leaves and feeder roots (Figure 3). A large portion of the amount which showed up in feeder roots was probably due to contamination of the sample by

Table 3. Mean carbohydrate % over time (harvest dates 1-8 = Aug, 16; Sept, 5; Nov, 1; Nov, 15, 1982 and Jan, 31; March, 21; June, 2; 1983, respectively) for whole plant (TC%) and plant parts (LC%, SC%, RC%, FRC% = leaf, stem, structural root and feeder root carbohydrate %, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 = medium P; 3 = high P) and either inoculated (I) or uninoculated (UI) with the vesicular-arbusculalr endomycorrhizal fungus Glomus etunicatum

		Harvest Date								
	Treatment	1	2	3	4	5	6	7	8	SEM[a]
	1	5.7	8.2	8.0	-[b]	-	-	-	2.4	
LC%	2	4.4	5.7	4.5	-	-	-	-	3.4	1.1
	3	4.8	5.9	4.4	-	-	-	-	1.7	
	1	8.5	15.6	19.6	13.5	10.2	5.5	6.9	3.4	
SC%	2	5.6	6.6	16.0	12.5	8.2	3.5	5.2	4.8	1.5
	3	7.6	8.2	13.2	12.4	8.6	3.9	3.2	3.2	
	1	0.15	2.1	5.7	7.5	4.0	2.6	2.6	0.92	
FRC%	2	0.00	1.5	4.5	6.2	3.2	1.5	0.50	0.26	0.82
	3	0.84	1.8	4.3	7.4	3.0	1.8	2.4	0.74	
	1	13.2	29.8	53.8	31.8	35.7	29.1	24.2	7.4	
RC%	2	11.3	19.4	43.4	36.7	27.2	18.4	24.5	12.9	3.4
	3	8.7	21.3	41.5	36.5	28.9	22.0	19.1	13.4	
	I	11.3	22.7	45.0	32.4	21.0	23.0	22.4	11.8	
RC%	UI	10.3	23.5	47.5	37.8	39.5	23.5	22.6	10.6	2.8
	1	7.3	15.8	23.4	17.3	16.6	12.7	11.2	3.9	
TC%	2	5.6	9.0	21.3	18.5	12.9	7.8	10.2	5.6	1.3
	3	5.7	10.5	19.7	18.7	13.5	9.1	8.6	5.8	
	I	6.6	11.6	22.2	17.9	10.9	9.2	9.7	5.4	
TC%	UI	5.9	11.4	22.7	18.5	17.5	9.9	10.4	5.0	1.1

[a]SEM = standard error for the difference between 2 means.

[b]No leaves due to dormant season.

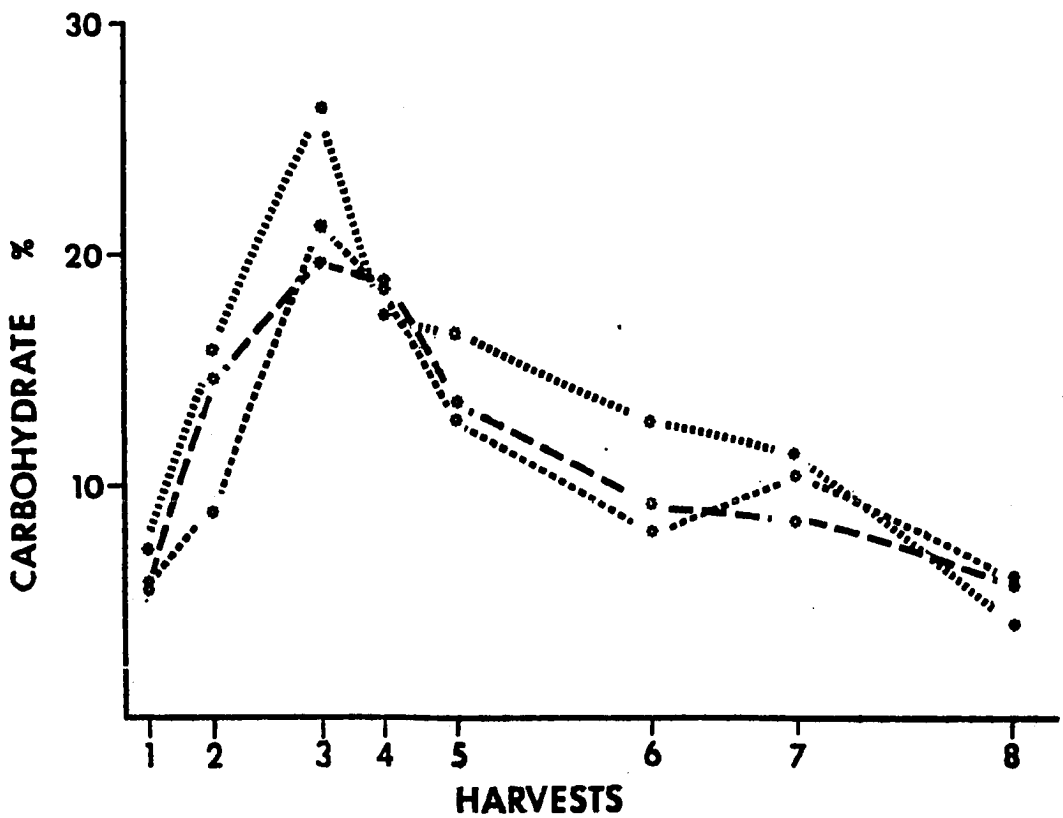

Figure 2. Mean carbohydrate percent for sugar maple (Acer saccharum Marsh) seedlings grown at three soil phosphorus (P) levels (····· = low P; ▪▪▪▪ = medium P; ▪—▪ = high P) over time (harvest dates 1-8 = Aug. 16; Sept. 5; Oct. 10; Nov. 1; Nov. 15, 1982 and Jan. 31; Mar. 21; June 2, 1983, respectively)

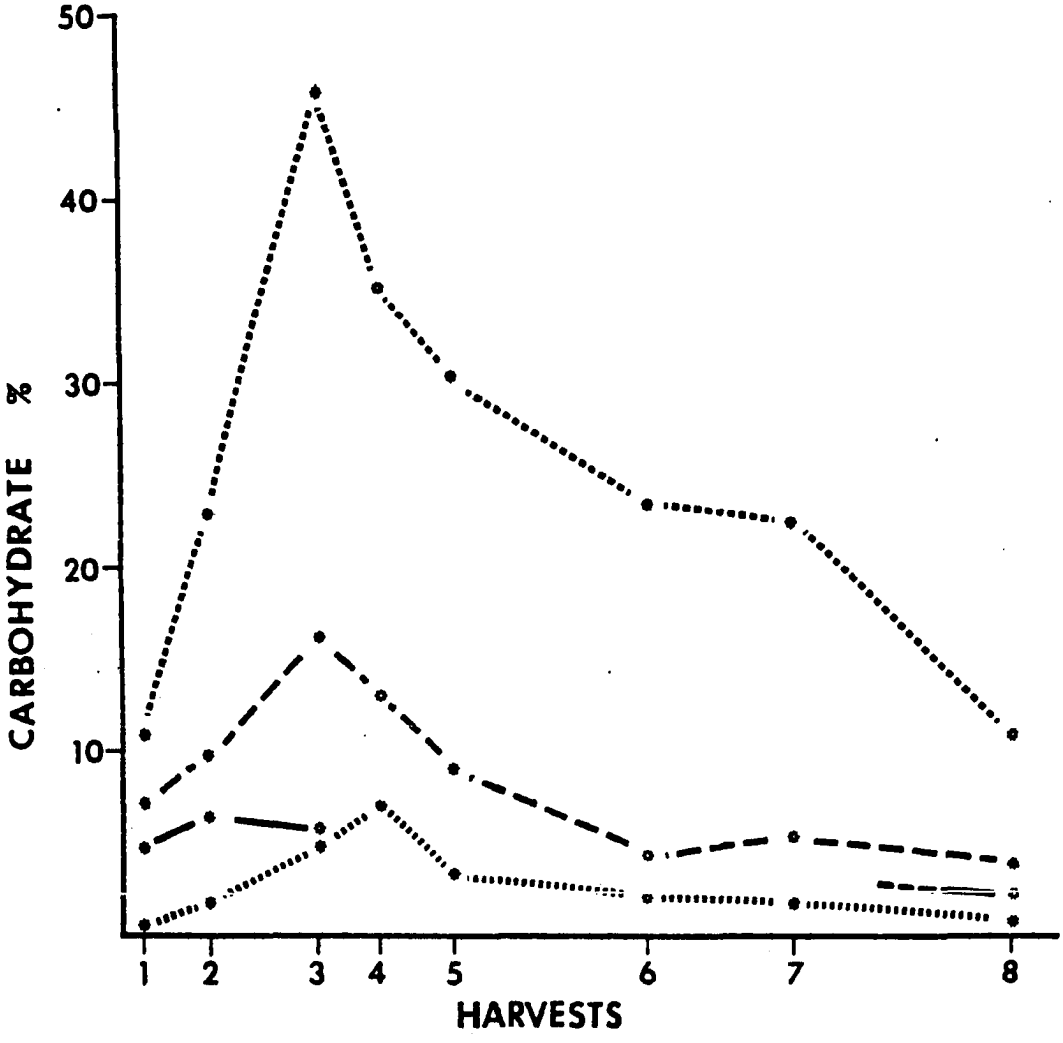

Figure 3. Mean carbohydrate percent for plant parts of sugar maple (Acer saccharum Marsh) (leaves = ——— ; stems = - - - ; roots = ••••••• ; feeder roots = •••••••) over time (harvest dates 1-8 = Aug. 16; Sept. 5; Oct. 10; Nov. 1; Nov. 15, 1982 and Jan. 31; Mar. 21; June 2, 1983, respectively)

pieces of structural roots since complete separation of the spongy freeze dried material was difficult. The largest amount of carbohydrate, by far, occurred in structural roots, regardless of harvest date, with a maximum of 46.3 percent at harvest 3. Stem carbohydrate levels were 2 and 3 times higher than leaf and feeder root levels, respectively, but were 3 times lower than the levels found in structural roots. When carbohydrate data were separated by plant parts, trends similar to those observed for total carbohydrate were found in each case. An interesting exception is the effect of mycorrhizal inoculation, that was observed with total carbohydrate data, but was not apparent when leaves, stems, and feeder roots were analyzed separately. The effect of mycorrhizal inoculation was significant ($P = 0.005$) for structural root data, however, where inoculated seedlings had 19 percent less carbohydrate at harvest 5 (Figure 4). Therefore, the ability to identify this trend with whole plant carbohydrate percent can be attributed, specifically, to the effect of inoculation on root carbohydrate levels.

It is well-known that woody plants accumulate carbohydrate reserves in times of excess production, reaching a maximum in fall with deciduous trees in temperate zones (Kramer and Kozlowski, 1979). The general pattern of slow decrease in reserve carbohydrates in late winter followed by a rapid decrease with spring regrowth was observed with sugar

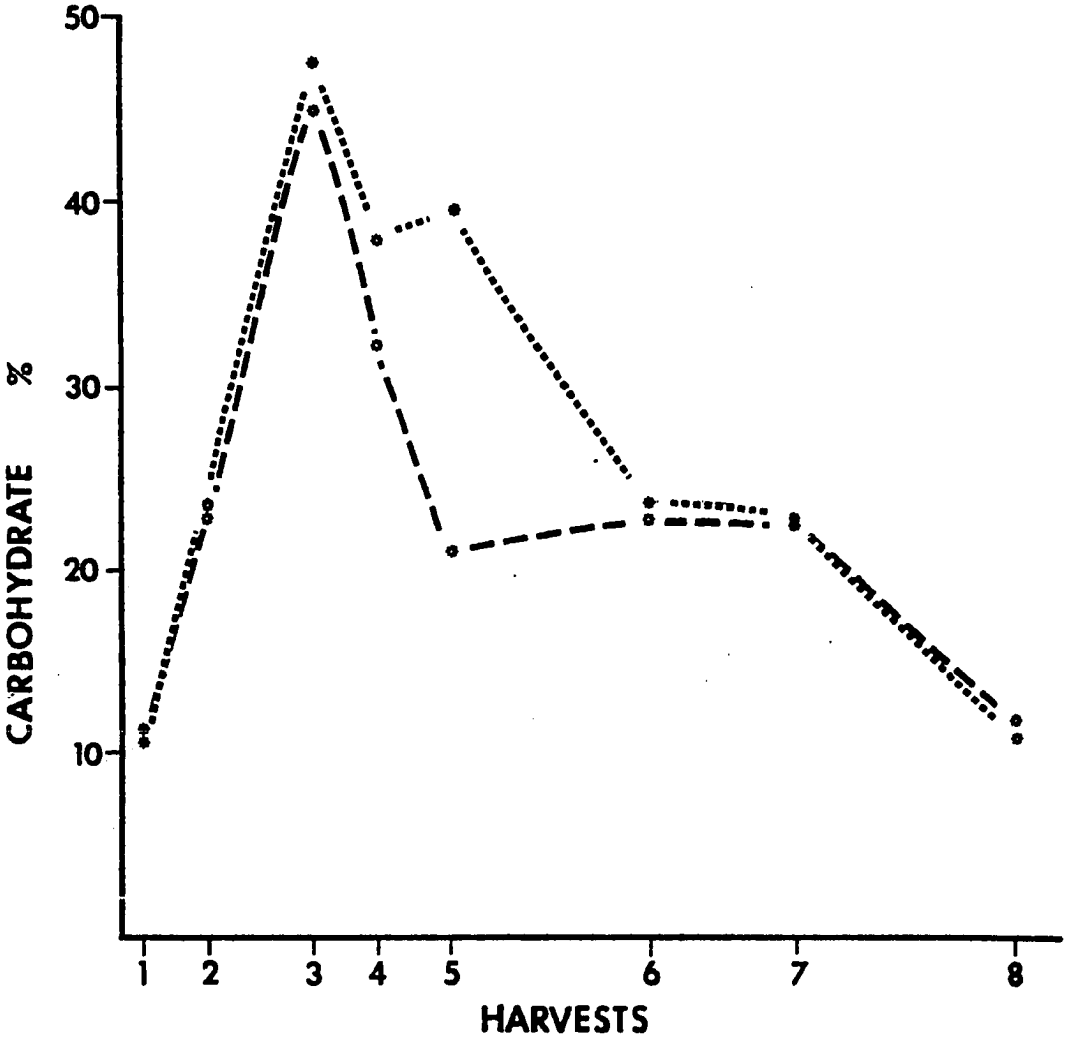

Figure 4. Mean carbohydrate percent for inoculated (- — -) and uninoculated (·····) sugar maple (Acer saccharum Marsh) seedlings over time (harvest dates 1-8 = Aug. 16; Sept. 5; Oct. 10; Nov. 1; Nov. 15, 1982 and Jan. 31; Marc. 21; June 2, 1983, respectively)

maple in Vermont by Jones and Bradlee (1933). The most rapid decrease observed by these workers occurred between February and April and may have been associated with a surge in root growth during this time. Taylor and Dumbroff (1975) and Dumbroff and Brown (1976) found that root growth in sugar maple continued throughout the dormant season, ceasing only when the ground was frozen in December and January. A surge in root growth was observed from late February through early April. This was followed by an increase in cytokinin activity that peaked just prior to bud break and was highest in lateral roots. In the present study, a rapid decrease in carbohydrate levels was observed in spring between harvests 7 and 8. From the above information, one would expect to observe a rapid decrease in the level of reserves beginning at harvest 6, with February root growth. This was not the case, however, and was probably related to the fact that soil in the pots was frozen for extended periods between harvests 5 and 7. The most rapid decrease in plant carbohydrate occurred between harvests 3 and 5 and was due primarily to changes in root levels because they contained such a large portion of the plant's reserve. In addition, most of the effect on root levels was due to the influence of inoculation on root carbohydrate percent between harvests 4 and 5 which was quite striking. This may have been due to accumulation of reserves in spores and extramatrical hyphae (Mosse, 1959; Mosse and Bowen, 1968; Ho and Trappe,

1973; Cooper, 1976) by the fungus in preparation for the winter season. These structures are left behind when the roots are extracted from soil for analysis.

Mycorrhizal inoculation was responsible for substantial levels of infection which varied with soil P and harvest date (Table 4). Uninoculated seedlings were completely free from infection in nearly every case and the few seedlings, where accidental infection was observed, were deleted from the data set. There was a strong direct relationship between infection percent (IP) and infection intensity (II). These infection parameters increased with harvest date (IP: $P = 0.002$; II: $P = 0.06$) from 4.6 and 18.7 at harvest 1, for IP and II, respectively, to maximum levels of 15.8 and 38.6 at harvest 3. Steady decreases, that continued through harvest 8, occurred and resulted in values of 4.1 (IP) and 17.0 (II). Soil P had a strong influence on infection (IP, II: $P = 0.0001$) with the lowest levels occurring at MP with mean values of 2.3 (IP) and 10.5 (II). Relatively moderate levels occurred at HP (IP = 7.4; II = 25.3) and the highest levels were observed at LP (IP = 15.1; II = 41.4). At a greater level of breakdown (Table 4), the highest mean values of 27.9 (IP) and 66.0 (II) occurred at harvest 3 in the LP treatment.

Infection levels were relatively low in the study even at low soil P. The factors that determine the level of infection are numerous and interact in a complex manner (Hayman, 1983).

Table 4. Mean infection percent (IP) and infection intensity (II) over time (Harvest dates 1-8 = Aug, 16; Sept, 5; Oct. 10; Nov, 1; Nov, 1; Nov, 15, 1982 and Jan, 31; March, 21; June, 2; 1983, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 = medium P; 3 = high P) and either inoculated (I) or uninoculated (U() with the vesicular-arbuscular endomycorrhizal fungus Glomus etunicatum

		Harvest Date								
	Treatment	1	2	3	4	5	6	7	8	SEM[a]
IP	1	8.4	24.6	27.9	16.0	16.0	12.4	6.1	8.3	5.3
	2	0.2	6.1	2.9	2.6	0.5	3.5	1.4	1.5	
	3	5.1	9.3	15.8	10.2	3.0	2.6	10.4	2.8	
II	1	35.0	46.0	66.0	31.1	51.5	42.9	28.8	22.3	7.8
	2	3.0	24.6	2.8	7.7	10.7	13.2	8.8	12.0	
	3	8.1	22.6	41.7	34.9	19.1	9.6	39.1	16.9	

[a]SEM = standard error for the difference between 2 means.

The level of infection is commonly unrelated to growth responses observed in the host (Kormanik et al., 1981; Bethlenfalvay et al., 1982a; Graham et al., 1982). An efficient symbiont could improve P nutrition and growth and at the same time utilize a small amount of host photosynthate at levels well below the 28% recorded at harvest 3 at LP. Therefore, one cannot conclude that the basic lack of a growth response was due to an inadequate level of infection. Since P nutrition was improved at LP by inoculation and since a substantial carbohydrate drain of 19% was observed at harvest 5, it is more likely that an inefficient or inappropriate symbiont was used.

The seasonal pattern of infection which was highest in the fall at harvest 3, was similar to those observed by Gay et al. (1982) in biennial and perennial species of closed chalkand turf. These workers observed maximum infection in the autumn of the first season and during the second summer of growth. They observed a high proportion of lysed hyphae during the first autumn and at the end of the second summer indicating that the most active infections occurred earlier during the period of most rapid growth and accumulation of N, P, and K by the host. The low level observed at harvest 8 in the present study is unexpected and may be further evidence that the relationship was inappropriate.

The occurrence of the lowest infection levels at MP is an

interesting result. One would expect a depression in the amount of infection with soil P level up through the HP level of 195 mg/kg found in the study. Why this anticipated effect of soil P level on infection was greater at MP is difficult to explain. The highest level of P fertilization was associated with significantly reduced levels of extractable soil K, Ca, Fe, and Zn (Table 5). Infection may have been encouraged, in view of the low levels, representing an indirect effect of P fertilization.

All treatments had significant effects on nutrient levels in plant parts (Tables 6, 7, and 8). Mycorrhizal inoculation influenced the levels of Mg, Fe, and Zn in structural root tissue ($P = 0.039$, 0.068, and 0.0006, respectively). Soil P level had highly significant ($P > 0.01$) effects on the levels of leaf N, P, K, Ca, and Zn, structural root N, P, K, and Ca and feeder root P, K, Ca, Mg, and Fe. A soil P effect on leaf Fe and feeder root N and Zn was also indicated ($P = 0.03$, 0.05, and 0.02, respectively). Harvest date had a significant effect on the levels of all nutrients in plant parts ($P > 0.001$) except leaf Fe.

Leaf N levels increased from a mean value of 2.7 percent at harvest 1 to a maximum of 3.3 percent at harvest 2 and decreased to 3.0 percent at harvest 3 just before leaf abscission ($P = 0.0001$). The next harvest with leaves was harvest 8, where the lowest level of 2.4 percent was observed.

Table 5. Mean soil nutrient levels (e.g., SP = soil phosphorus) in ppm, over time (Harvest Dates 1-8 = Aug, 16; Sept, 5; Oct, 10; Nov, 1; Nov, 15, 1982 and Jan, 31; March, 21; June, 2; 1983, respectively) at 3 levels of phosphorus (P) fertilization (1 = low P; 2 = medium P; 3 = high P)

		Harvest Date								
	PHOS	1	2	3	4	5	6	7	8	SEM[a]
	1	56	30	31	29	30	29	31	29	
SP	2	146	147	147	143	138	136	135	126	19
	3	188	194	193	195	190	191	200	194	
	1	89	120	112	110	121	107	117	116	
SK	2	84	103	95	93	94	106	98	97	5
	3	76	93	75	82	88	88	86	85	
	1	533	527	574	547	541	545	564	536	
Sca	2	558	560	547	553	557	516	531	555	55
	3	384	400	361	365	336	430	468	516	
	1	110	108	115	113	103	110	117	109	
SMg	2	104	104	104	104	106	100	101	106	4
	3	109	106	106	106	107	104	106	109	
	1	247	250	286	262	256	238	251	259	
SFe	2	173	185	188	194	197	184	196	177	25
	3	145	157	162	166	155	168	162	159	
	1	8.7	8.3	9.2	8.3	8.4	7.9	8.5	9.1	
SZn	2	6.7	8.6	7.6	8.2	8.2	6.5	7.2	7.8	0.8
	3	5.2	5.3	5.8	5.9	5.7	6.3	6.0	6.7	

[a]SEM = standard error for the difference between means.

Table 6. Mean nutrient levels in mg/g (in % for nitrogen) for leaf tissue (e.g., LN = leaf nitrogen) over time (Harvest dates 1-8 = Aug, 16; Sept, 5; Oct, 10; Nov, 1; Nov, 15, 1982 and Jan, 31; Mar, 21; June, 2, 1983, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 = medium P; 3 = high P) and either inoculated (I) or uninoculated (UI) with the vesicular-arbuscular endomycorrhizal fungus Glomus etunicatum

		Harvest Date				
	Treatment	1	2	3	8	SEM[a]
	1	2.5	2.9	2.7	2.4	
LN%	2	2.7	3.5	3.2	2.3	0.2
	3	2.7	3.3	3.2	2.7	
	1	1.1	1.1	1.1	1.1	
LP	2	3.6	4.2	5.3	2.9	0.6
	3	5.5	6.4	8.0	3.1	
	1	7.4	6.4	8.6	7.8	
LK	2	8.9	9.8	9.5	7.9	0.7
	3	9.7	8.8	9.9	7.6	
	1	12.5	10.6	12.4	8.7	
LCa	2	14.2	14.1	16.7	9.8	1.2
	3	15.9	14.8	19.5	9.8	
	1	1.12	0.93	0.86	1.12	
LMg	2	1.03	0.96	0.87	1.10	0.07
	2	1.13	0.92	0.86	1.11	
	1	0.22	0.24	0.21	0.17	
LFe	2	0.21	0.24	0.22	0.17	0.035
	3	0.16	0.21	0.13	0.17	
	1	0.071	0.031	0.054	0.032	
LZn	2	0.039	0.028	0.052	0.041	0.007
	3	0.034	0.024	0.045	0.039	

[a]SEM = standard error for the difference between 2 means.

Table 7. Mean nutrient levels for root tissue (e.g., RN = root nitrogen) over time (harvest dates 1-8 = Aug, 16; Sept, 5; Oct, 10; Nov, 1; Nov, 15, 1982 and Jan, 31; March, 21; June, 2, 1983, respectively for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 = medium P; 3 = high P) and either inoculated (I) or uninoculated (UI) with the vesicular-arbuscular endomycorrhizal fungus Glomus etunicatum in mg/g or in % for nitrogen

		Harvest Date								
	Treatment	1	2	3	4	5	6	7	8	SEM[a]
	1	1.7	2.2	1.8	2.3	2.4	2.6	2.9	1.9	
RN%	2	1.7	2.7	2.2	2.4	2.4	3.1	2.7	1.0	0.35
	3	2.0	2.8	2.4	3.1	2.7	3.2	3.6	1.4	
	1	1.1	1.2	1.2	1.3	1.2	1.3	1.3	0.4	
RP	2	3.2	3.6	3.0	2.9	3.0	3.3	3.0	2.0	0.30
	3	3.9	4.1	3.3	3.6	3.7	3.3	3.7	2.6	
	1	10.5	10.0	5.0	4.9	4.7	5.6	5.2	3.8	
RK	2	10.9	12.6	9.0	6.6	6.5	6.7	6.1	4.1	0.63
	3	13.5	13.3	7.7	8.8	8.2	5.9	5.5	3.7	
	1	3.5	2.2	1.8	2.3	2.0	2.1	2.3	3.4	
RCa	2	3.4	3.9	3.3	3.0	2.6	3.2	3.2	4.1	0.45
	3	4.3	3.9	3.3	3.6	4.0	3.6	3.6	4.5	
	1	0.41	0.47	0.47	0.38	0.47	0.38	0.36	0.22	
RMg	2	0.48	0.48	0.49	0.48	0.51	0.43	0.30	0.22	0.05
	3	0.55	0.60	0.45	0.52	0.58	0.34	0.37	0.21	
	I	-	0.53	0.52	0.45	0.52	0.44	0.36	0.26	
RMg	UI	0.48	0.52	0.42	0.48	0.52	0.32	0.33	0.18	0.04
	1	0	0.002	0.017	0.007	0.005	0.005	0.005	0.005	
RFe	2	0	0.010	0.006	0.006	0.007	0.005	0.005	0.005	0.005
	3	0	0.013	0.007	0.005	0.006	0.005	0.005	0.005	
	I	-	0.01	0.013	0.006	0.007	0.005	0.005	0.005	
RFe	UI	0	0.008	0.008	0.006	0.005	0.005	0.005	0.005	0.004
	1	0.006	0.008	0.018	0.010	0.013	0.011	0.008	0.025	
RZn	2	0.005	0.011	0.017	0.018	0.022	0.016	0.005	0.008	0.005
	3	0.009	0.013	0.014	0.017	0.019	0.008	0.010	0.006	
	I	-	0.011	0.020	0.014	0.019	0.016	0.007	0.020	
RZn	UI	0.006	0.010	0.012	0.017	0.019	0.008	0.010	0.006	0.004

[a]SEM = standard error for the difference between 2 means.

Table 8. Mean nutrient levels in mg/g (in % for nitrogen) for feeder root tisue (e.g., FRN = feeder root nitrogen) overtime (harvest dates 1-8 = Aug, 16; Sept, 5; Oct, 10; Nov, 1; Nov, 15; 1982 and Jan, 31; March, 21; June 2, 1983, respectively) for sugar maple (Acer saccharum Marsh) seedlings grown at 3 soil phosphorus (P) levels (1 = low P; 2 = medium P; 3 = high P) and either inoculated (I) or uninoculated (UI) with the vesicular-arbuscular endomycorrhizal fungus Glomus etunicatum

		Harvest Date								
Treatment		1	2	3	4	5	6	7	8	SEM[a]
	1	-[b]	2.7	3.0	3.3	3.0	2.6	2.4	1.5	
FRN%	2	-	2.5	2.6	2.7	2.6	2.3	2.4	1.4	0.35
	3	1.7	2.3	2..4	3.0	2.5	2.6	2.9	1.3	
	1	-	1.6	1.6	1.3	1.1	1.5	1.9	1.2	
FRP	2	-	5.8	5.8	6.1	4.8	3.5	3.7	3.1	0.31
	3	6.9	7.8	8.3	7.5	4.7	4.5	4.8	4.5	
	I	-	5.8	5.5	6.2	3.7	3.7	3.9		
3.9										
FRP	UI	6.9	6.7	5.9	4.9	4.0	3.4	3.5	3.0	0.26
	1	-	8.2	8.8	10.9	6.2	5.4	3.1	6.8	
FRK	2	-	14.2	14.9	19.4	11.1	6.2	5.6	6.3	0.26
	3	12.1	13.7	15.6	18.2	8.6	6.5	6.1	7.6	
	1	-	7.3	6.3	5.8	6.9	7.3	7.0	7.7	
FRCA	2	-	7.5	7.6	6.8	6.9	7.6	8.3	7.9	0.50
	3	8.2	8.8	7.3	6.1	8.1	8.4	7.9	7.7	
	1	-	1.9	1.7	1.30	0.8	0.6	0.8	0.7	
FRMG	2	-	2.8	2.8	2.2	1.5	0.8	0.7	0.9	0.21
	3	1.6	3.3	2.8	1.7	1.2	0.8	1.0	1.0	
	1	-	0.45	0.57	0.38	0.38	0.48	0.61	0.24	
FRFE	2	-	0.86	1.69	0.76	0.59	0.63	0.45	0.37	0.31
	3	0.51	0.17	0.91	0.39	0.58	0.40	0.66	0.26	
	1	-	0.09	0.17	0.16	0.06	0.05	0.05	0.07	
FRZN	2	-	0.17	0.23	0.18	0.13	0.05	0.04	0.08	0.03
	3	0.14	0.21	0.17	0.16	0.07	0.03	0.05	0.05	

[a] SEM = standard error for the difference between 2 means.

[b] Insufficient plant material for analysis.

This low level at harvest 8, which was also noted visually probably reflects a dilution of plant N due to rapid growth. The fact that the effect is greater at MP and HP than at LP ($P = 0.0005$) is evidence of N dilution due to growth (Table 6). Leaf N was higher in MP and HP seedlings for harvests 1-3 but, due to the lack of growth by LP seedlings and a subsequent lack of N dilution, LP seedlings had the highest leaf N at harvest 8. This effect, combined with the fact that leaf N levels for the different soil P treatments diverged from similar initial levels, accounts for the interactions among treatments that occurred for this variable. Nitrogen levels were of similar magnitude in structural roots (Table 7) and feeder roots (Table 8). Fluctuations in the N levels of these plant parts followed a pattern similar to leaf N data.

There was no effect of inoculation on plant N levels even though the uptake of N by mycorrhizal hyphae has been demonstrated with celery plants using ^{15}N in a split culture system (Ames et al., 1983). Nitrogen appears to be very important in the function of the symbiosis. Hall et al. (1984) suggested that applied N resulted in more efficient mycorrhizal uptake of P and Cu. Increasing rates of N fertilization resulted in higher levels of infection in lettuce roots (Hepper, 1983). This effect was observed at three P levels. At low P, the level of infection was highly dependent upon N level illustrating the potential for complex

interactions. Supplemental N was applied at the rate determined to be optimal for the growth of mycorrhizal sweetgum seedlings (Brown et al., 1981). This resulted in N levels in sugar maple seedling tissue that were higher than those commonly observed with sweetgum seedlings. It is conceivable that the maple seedlings maintained this higher level at the expense of size growth, which was less than that observed with sweetgum. This would, therefore, indicate that insufficient N was present for the proper nutrition of sugar maple seedlings and the full expression of a mycorrhizal effect of P uptake.

The relationship between soil P and the level of P in plant parts was direct and clearly expressed. Phosphorus levels generally increased in plant parts during the first growing season and decreased thereafter. Feeder roots contained the highest levels of P with an average of 4.5 mg/kg followed by leaves and structural roots which averaged 3.7 and 2.5 mg/kg, respectively. The greatest fluctuations in plant P also occurred in feeder roots. At LP, feeder roots averaged only 1.5 mg/kg and exhibited little variation with harvest date. Large variations were observed at MP and HP in feeder roots where P levels averaged 4.6 and 6.0 mg/kg, respectively ($P = 0.0001$). Feeder root P levels increased early in the study and reached 8.3 mg/kg for HP seedlings at harvest 3 and 6.1 mg/kg for MP seedlings at harvest 4 ($P = 0.0001$). An

extremely sharp decrease occurred in feeder roots between harvests 4 and 5, at both of these soil P levels, as the seedlings became dormant. A slight downward trend was observed for all 3 P levels between harvests 7 and 8. Similar variations occurred in structural roots except for the sharp decrease during the first fall, which occurred earlier, between harvests 2 and 3.

Leaf P levels increased with harvest ($P = 0.0001$) to harvest 3, where they averaged 4.8 mg/kg. The leaves at harvest 8 had the lowest level (2.3 mg/kg) of any study harvest. Leaf P was highly dependent upon soil P level ($P = 0.0001$) with an average of 1.1 mg/kg at LP, 4.0 mg/kg at MP and 5.9 at HP. The harvest by soil P level interaction ($P = 0.0001$) can be attributed to the lack of variation at LP with harvest date relative to MP and HP. There was no main effect of mycorrhizal inoculation on leaf P but the mycorrhizal inoculation by harvest date interaction was highly significant ($P = 0.0004$). At harvest 3, leaf P levels were substantially greater for inoculated seedlings than for uninoculated seedlings ($P = 0.0004$). This was true for all 3 soil P levels. The difference in leaf P between inoculated and uninoculated seedlings at harvests 2 and 3 was apparent visually, as described earlier, for LP seedlings.

Phosphorus was clearly more available at HP resulting in an average total plant uptake of 7.5 mg of P compared to 3.7

and 2.2 mg for MP and LP seedlings, respectively. Therefore, a lack of P at HP did not exist and cannot explain depressed growth at this level relative to MP. Conversely, these levels are extremely high relative to the sweetgum system. This could support the idea presented above that the levels accumulated at HP are directly inhibitory.

Plant K averaged 10.2mg/kg for feeder root tissue, 6.9 mg/kg for structural roots and 8.6 mg/kg for leaves. Leaf K was dependent on harvest date ($P = 0.0002$), soil P level ($P = 0.0001$) and their interaction ($P = 0.0002$).

Feeder root K varied with harvest date ($P = 0.0001$) and was positively related to soil P. The interaction of soil P level and harvest date for feeder root K mirrored that which occurred for leaf K.

Root K levels were negatively related to harvest date ($P = 0.0001$) and were positively related to soil P level ($P = 0.0001$). The effect of soil P level was more pronounced for uninoculated seedlings resulting in a significant mycorrhizal inoculation by harvest date interaction ($P = 0.0001$).

The occurrence of the highest levels of P and K in feeder roots indicates the importance of considering this plant part as a distinct physiological tissue. Tissue analyses that consider the root as a collective organ tend to mask feeder root ditferences as would be the case with leaves if analyzed with stems.

As mentioned above, variations in the levels of Ca, Mg, Fe, and Zn in plant parts with treatments were also observed as seen in Tables 6-8. A detailed discussion of thetrends that exist in this data is available upon request. Reports on the effect of VAM symbiosis on the uptake of K, Ca, Mg, Fe, and Zn have not been consistent. All have been shown to be increased by infection under certain conditions, however (Cooper and Tinker, 1978; Gerdemann, 1964; Jensen, 1982; Thodes and Gerdemann, 1978; Rhodes and Gerdemann, 1980; Ross, 1971; Ross and Harper, 1971).

CONCLUSIONS

Even though a high degree of genetic variation was observed with the half-sib seed, responses to study treatments could be detected. The most interesting result was the lack of a mycorrhizal response with seedling size parameters. Mycorrhizal inoculation did result in substantial levels of infection, especially at LP, and did account for significantly higher levels of plant P, a result that was evident visually. In addition, inoculation had an effect on the levels of certain other elements in the seedlings, plant carbohydrate percent and root to shoot ratios. In view of this, it appears that mycorrhizal inoculation had an effect on seedling physiology. The complete lack of height growth at LP indicates that a threshold level of plant P necessary for shoot extension was not reached. The fact that mycorrhizal seedlings had higher leaf P at LP may indicate a carbohydrate drain on the host by the fungus since this increased level was not translated into growth. Direct evidence of a carbohydrate drain was observed at harvest 5 where roots of inoculated seedlings were 19% lower in carbohydrate percent than uninoculated roots. Alternately, this higher level of P may still have been below a threshold level for shoot extension even without a significant carbohydrate drain. It is possible that a more appropriate symbiont, in terms of species or isolate, may have helped the seedlings surpass this level in

the unamended 33 ppm study soil.

Sugar maple has been known to require high nutrition for some time (Post, 1968). This appears to be the case in the present study as the seedlings acquired substantial levels of nutrients in plant parts. Nutrient analysis indicates that feeder roots are distinct physiologically and contained much higher levels of nutrients than structural roots and in several cases higher levels than leaves.

Sugar maple are heavily mycorrhizal under natural conditions as observed by the authors in field collected roots from Vermont and Wisconsin. The study conditions were likely quite different from the natural situation since central Iowa is not included in the natural range of this species. The results do indicate an effect of VAM symbiosis that requires further investigation using different symbiont-environment interactions.

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GENERAL SUMMARY AND CONCLUSIONS

Sugar maple seedlings form a mycorrhizal symbiosis with vesicular-arbuscular endomycorrhizal (VAM) fungi. The degree of dependancy on the symbiosis expressed by this species is difficult to say since dependence is relative. The classic situation with other hardwood tree seedlings is characterized by a positive response to VAM symbiosis under conditions of low phosphorus (P) fertility. Effects on the host plant are not usually limited to this simple response, however. The host-endophyte system is complex and, to some degree, involves nearly all aspects of the physiology of both partners. Mycorrhizae improved the P nutrition of sugar maple seedlings at low soil P under the study conditions. The fungi utilized host produced photosynthate and altered the levels of various nutrients in plant tissues in addition to P. Therefore, the symbiosis did have an effect on sugar maple seedling physiology in the research presented here. The improved P nutrition was not translated into growth, however. Many possible explanations exist. Perhaps the species, although heavily mycorrhizal in nature, has a low dependancy on the symbiosis and the improved P nutrition was not that significant in terms of a possible internal threshold level that must be overcome for shoot extension. Recent evidence has indicated the importance of the particular fungal species or isolate in determining the result of the interaction in a

particular set of environmental conditions. A positive response is observed in the host, only when benefits in terms of improved P nutrition outweigh the photosynthate drain of the fungus. An inefficient symbiont could conceivably have a high metabolic rate and a poor ability to supply the host with P, making it expensive in terms of photosynthate use. The difference of 19% in root carbohydrate levels at harvest 3 in low P soil indicates that this may have been the case with the particular host-endophyte-system studied.

Anatomical investigations with this species determined a slightly different pattern of arbuscule initiation or possibly a different interpretation of information than has been reported with other systems. In addition, the unique beaded morphology of sugar maple roots was involved. Overall, however, anatomy of VAM infections appears to be determined primarily by the invading endophyte and does not vary appreciably even when comparing woody plant hosts with herbaceous plant hosts.

Sugar maple is a very complex species and requires extremely high nutrition evidenced by the relatively high tissue concentration of plant nutrients detected in study seedlings. Further, highly controlled factorial investigations will be required in order to begin to understand this plant. One of the first factors that should be considered is the level of nitrogen fertilization since it

is so important in the expression of the mycorrhizal response to poor P nutrition and since much higher levels were accumulated in the plant than anticipated when calculations for nitrogen fertilization were made. This holds for the levels of other nutrients that were found to be high in seedling tissues as well.